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# A THIRD CASE OF COMPENSATORY RECOMBINATION IN INTERSPECIFIC HYBRIDS OF GOSSYPIUM[1]

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Received June 30, 1961

THERE are now several cases reported in the literature in which the transfer of a chromosomal segment from one species of Gossypium to another results in altered recombination frequency in the chromosome to which the "foreign" segment has been transferred (SILOW 1944; RHYNE 1958; PHILLIPS 1961; STEPHENS 1961). Further, it is known that the altered recombination may take the form of either increased or decreased map distance (RHYNE 1958) and that a decrease in the region including the inserted segment may be more or less exactly compensated by an increase proximal to the inserted segment (STEPHENS 1961). This type of compensation is not confined to the chromosomes of species known to be cytologically differentiated, but also occurs between chromosomes which exhibit apparently normal pairing behavior during the meiotic cycle, and whose products of recombination appear to be completely viable (i.e. complete fertility of the $F_1$ hybrid). The present case is concerned with recombination in the leaf shape–crinkle–green lint linkage group of *Gossypium hirsutum* L. into which has been inserted by backcrossing a segment including the leaf shape locus from the related wild American species, *G. thurberi* Tod.

The linkage group has been described previously by STEPHENS (1955). He found that leaf shape (*L*) and crinkle (*cr*) are 41.2 map units apart, and that the map distance between crinkle (*cr*) and green lint (*Lg*) is 6.6. These map distances were based on recombinations obtained in intervarietal crosses of *hirsutum*. The diploid species, *G. thurberi*, carries a "homologous" chromosome which exhibits preferential pairing with its *hirsutum* counterpart in appropriate synthetic polyploid combinations (GERSTEL and PHILLIPS 1958). The narrow leaf of *thurberi* is known to be an allele of the leaf shape series in *hirsutum* (GREEN 1953), so that one might expect that the *thurberi* and *hirsutum* chromosomes would be homologous in the *L* region. GILES (in press) found that the *thurberi* chromosome does not carry a normal allele (*Cr*) of *hirsutum* crinkle (*cr*) which may indicate that the *thurberi* and *hirsutum* chromosomes are nonhomologous in this region. The seeds of *G. thurberi* bear no true lint; seeds of the synthetic amphiploid, *G. arboreum* × *G. thurberi*, bear a greyish-white fiber if the *arboreum* parent is white-linted. In crosses between *G. hirsutum* types with green lint (*Lg*) and the synthetic amphiploid, *arboreum* × *thurberi*, with grey-

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ish-white fiber, green lint behaves as a simple dominant over white (GILES, in press). This suggests either that the *thurberi* chromosome carries a recessive allele (*lg*) or that the green lint locus is missing entirely from this species.

The source material for this study was a trispecies hybrid, (*G. arboreum* × *G. thurberi*) × *G. hirsutum*, whose synthesis and constitution have been reported previously (GILES, in press). The *hirsutum* parent, SM2, carried the linked combination, *l—Cr—Lg*. The trispecies hybrid was backcrossed to a recessive marker stock of *hirsutum*, SM3, which carried the linked combination, *l—cr—lg*. From this backcross generation four plants with narrow leaf ($L^u$ from *thurberi*), noncrinkle and green lint phenotype were selected and backcrossed again to the recessive tester, SM3. The constitution of the selected plants should therefore be:

$$\frac{L^u \quad Cr \quad Lg}{l \quad cr \quad lg}$$

where the broken lines indicate *hirsutum* chromosomal segments and the solid line the inserted segment from *thurberi*.

From the selected plants four small second backcross families were raised. One of these was deficient in green lint segregates and was not used in the linkage calculations. The segregations obtained in the other three families are presented in Table 1.

A contingency test showed that the segregations were not homogeneous, although the individual gene segregations did not differ significantly from Mendelian expectation. The major source of heterogeneity was the recombination

TABLE 1

*Linkage analysis of three second backcross families of the trispecies hybrid,* (arboreum × thurberi) × hirsutum, *backcrossed to* hirsutum

| Genotype | Second backcross families G2-21 | G2-44 | G2-55 | Total |
|---|---|---|---|---|
| $L^u$–*Cr*–*Lg* | 9 | 10 | 14 | 33 |
| *l*–*cr*–*lg* | 12 | 16 | 11 | 39 |
| *l*–*Cr*–*Lg* | 3 | 7 | 12 | 22 |
| $L^u$–*cr*–*lg* | 0 | 2 | 3 | 5 |
| $L^u$–*Cr*–*lg* | 2 | 1 | 4 | 7 |
| *l*–*cr*–*Lg* | 0 | 0 | 2 | 2 |
| $L^u$–*cr*–*Lg* | 0 | 0 | 0 | 0 |
| *l*–*Cr*–*lg* | 6 | 0 | 2 | 8 |
| Total | 32 | 36 | 48 | 116 |

$\chi^2(12) = 21.35$, $P = 0.02$–$0.05$

| Family | Percent crossing over $L^u$–*Cr* | *Cr*–*Lg* |
|---|---|---|
| G2-21 | 28.13 ± 7.9 | 25.00 ± 7.7 |
| G2-44 | 25.00 ± 7.2 | 2.78 ± 2.7 |
| G2-59 | 35.42 ± 6.9 | 16.67 ± 5.4 |
| Total | 30.18 ± 4.3 | 14.66 |

frequency in *Cr—Lg* region. In order to determine the nature of this heterogeneity single plants carrying the appropriate dominant markers were selected from Families G2-21 and G2-44 and backcrossed a third time to the recessive tester, SM3. Their segregations in the *Cr—Lg* interval are analyzed in Table 2. It can be seen that the two families segregated homogeneously in the third backcross generation and that their joint crossover percentage agrees reasonably well with the average crossover percent obtained in the previous generation (Table 1).

In Table 3, the recombination frequencies obtained in the interval, *l—lg*, for the data presented here are compared with the estimates obtained earlier (STEPHENS 1955) from intraspecific crosses. It can be seen that the insertion of a *thurberi* segment in the region of the *l* locus has reduced recombination from 41.2 percent to 30.2 percent in the *l—cr* interval. In the neighboring interval, *cr—lg*, in which no *thurberi* segment is involved, there is a compensatory increase from 6.6 percent to 15.9 percent. The total map length from *l* to *lg* remains essentially unchanged (47.8 in the intraspecific data as compared with 46.1 in the data from the interspecific cross reported here). This suggests that the total chiasma frequency has not been reduced in the interspecific cross, but that the position in which the chiasma most frequently occurs has been shifted as the result of inserting a "foreign" segment. Thus, although the genetic evidence obtained by GREEN (1953) indicates that *thurberi* and *hirsutum* possess a leaf shape locus in common, the cytogenetic evidence presented here suggests that the chromosomal segments in which the common locus occurs are not strictly homologous.

TABLE 2

*Linkage analysis of two third backcross families of the trispecies hybrid,* (arboreum × thurberi) × hirsutum, *backcrossed to* hirsutum

| Family | *Cr Lg* | *Cr lg* | *cr Lg* | *cr lg* | Total |
|---|---|---|---|---|---|
| G2-21 | 24 | 5 | 10 | 42 | 81 |
| G2-44 | 44 | 6 | 9 | 48 | 107 |
| Total | 68 | 11 | 19 | 90 | 188 |

$\chi^2(3) = 3.03$, P = 0.30–0.50

Combined crossover percent = 15.86 ± 2.7

TABLE 3

*A comparison of intra- and interspecific recombination in the segment,* l—cr—lg. *(Intraspecific data from* STEPHENS *1955)*

| Interval:– | *l—cr* | *cr—lg* | Total |
|---|---|---|---|
| Intraspecific | 41.2 ± 3.1 | 6.6 ± 3.2 | 47.8 |
| Interspecific | 30.2 ± 4.3 | 15.9 ± 2.7 | 46.1 |

SUMMARY

A case of compensatory recombination in the segregating progenies of interspecific hybrids in Gossypium is described. The insertion of a chromosomal segment, marked by a leaf shape gene ($L^u$), from *G. thurberi* into the "homologous" chromosome of *G. hirsutum*, produces a change in recombination pattern. Crossing over in the inserted segment is reduced from 41.2 to 30.2 percent, and increased in the neighboring *cr—lg* segment from 6.6 to 15.9 percent. Recombination in the total mapped region from *l* to *lg* remains virtually constant. The insertion of the "foreign" segment apparently results in a shift in chiasma position.

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# COMPLEMENTATION AT THE YELLOW LOCUS IN DROSOPHILA MELANOGASTER

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Received August 21, 1961

AS a general rule allelic or pseudoallelic mutants when compounded produce either an intermediate phenotype or one equivalent to the lesser departure from wild type. Cases have been infrequently described where the compound of presumed alleles manifests a complementary phenotype, i.e. one more normal than that of either allele. By and large allelic complementation in Drosophila has been sporadic and has not seriously challenged the unitary concept of the gene. One noteworthy exception has been the complementary phenotypic effects associated with the various scute bristle (*sc*) mutants, effects which formed the basis of the step-allelomorph theory of genic subdivision. Although invalidated, step-allelomorphism has its current counterpart in the complementation alleles and complementation maps so thoroughly developed in Neurospora by GILES, by FINCHAM and by CATCHESIDE and their several collaborators.

## EXPERIMENTAL RESULTS

Two general phenotypic classes of sex-linked, recessive yellow body color ($y$) mutants have been described in *Drosophila melanogaster*. One, typified by the original $y$ mutant, manifests a yellow body and yellow bristle phenotype. The second, of which the mutant $y^2$ is typical, manifests a yellow body but black bristle coloration. STONE (1935) described a unique example of complementation at this locus. He observed that the mutant $y^{35a}$, a $y$-type X-ray induced in *In(1)99b*, produced when compounded to $y$ the usual $y$ phenotype but when compounded to $y^2$ resulted in a complementary, wild-type phenotype. The heterozygote $y/y^2$ is phenotypically equivalent to homozygous $y^2$. Unfortunately $y^{35a}$ was lost before a more detailed study could be pursued.

A re-examination and extension of complementation among $y$ mutants was made possible by the chance finding that the spontaneous mutant $y^{8i}$ which occurred in the $In(1)sc^{8l}$ chromosome had complementation properties identical to $y^{35a}$. The complex X-chromosome $y^{8i}In(1)sc^{8i}$ *In(1)*dl-49 *B v* (*B*=Bar eye, *v*= vermilion eye color) obtained from PROFESSOR H. J. MULLER for use as a balancer chromosome was found, as listed in Table 1, to produce a near wild-type phenotype when compounded with $y^2$ but not to the other $y$ mutants to which it was tested. Independently, FRYE (1960) also uncovered the complementary interaction of $y^{8i}$ and $y^2$.

A second $y$ type mutant of rather unusual origin, $y^{59b}$, was found to complement with $y^2$ and with none of the other $y$ mutants to which it was tested as

TABLE 1

*Phenotype of* y *mutant compounds*

| *y* mutant | *y* | $y^2$ | $y^{59b}$ | $y^{2p}$ | $y^{31d}$ | $y^{50k}$ | $y^{51g}$ | $y^{60b}$ | $y^{60j}$ | $y^{8i}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| *y* | *y* | $y^2$ | *y* | $y^2$ | *y* | *y* | $y^2$ | *y* | *y* | *y* |
| $y^2$ | | $y^2$ | $y^+$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^+$ |
| $y^{59b}$ | | | *y* | $y^2$ | $y^2$ | *y* | $y^2$ | *y* | *y* | *y* |
| $y^{2p}$ | | | | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ |
| $y^{31d}$ | | | | | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ | $y^2$ |
| $y^{50k}$ | | | | | | *y* | $y^2$ | *y* | *y* | *y* |
| $y^{51g}$ | | | | | | | $y^2$ | $y^2$ | $y^2$ | $y^2$ |
| $y^{60b}$ | | | | | | | | *y* | *y* | *y* |
| $y^{60j}$ | | | | | | | | | *y* | *y* |
| $y^{8i}$ | | | | | | | | | | *y* |

tabulated in Table 1. The origin of $y^{59b}$ briefly described previously (GREEN 1960) merits repetition here. Males of the genotype $y^2$*su-w*$^a$*z* (*su-w*$^a$ = suppressor of white-apricot, *z*= zeste eye color) irradiated with 5,000r X-rays were crossed to females homozygous for the complex X chromosome inversions *sc*$^s$ and dl-49 and the recessive markers *y*, *w*$^a$, *v* and *f* (forked bristles). Among approximately 150,000 $F_1$ females scored, one wild-type female was found. On progeny testing this female produced three types of males in approximately the following frequencies: 50 percent *y w*$^a$*v f*, 25 percent $y^{59b}$*su-w*$^a$ *z* and 25 percent $y^+$ *su-w*$^a$ *z*. Thus two events followed irradiation: the reversal of $y^2$ to $y^+$ and the mutation of $y^2$ to $y^{59b}$. Subsequent genetic tests established the presence of both *su-w*$^a$ and *z* in both the mutated X chromosomes. Insofar as can be determined by genetic tests, $y^{59b}$ is devoid of any structural change. Crossing over between $y^{59b}$ and the tightly linked *su-w*$^a$, about 0.2 percent to the right, occurs freely.

Of particular interest is the finding that the complementation potential of $y^2$ is confounded by the mutant *sc*. While compounds of $y^2$, derived from a variety of stocks, and $y^{8i}$ or $y^{59b}$ were without exception complementary, those of coupled $y^2$*sc* and $y^{8i}$ or $y^{59b}$ were noncomplementary and were indistinguishable from homozygous $y^2$. As a test of the specific influence of *sc* upon $y^2$, thirteen independent phenotypically complete and one partial X-ray induced reversals of *sc* induced in $y^2$*sc* X chromosomes (GREEN 1961) were employed. Where each $y^2$ complete *sc* reversal was compounded with $y^{8i}$ or $y^{59b}$, the complementary phenotype was restored. Conversely, the compound of $y^2$ partial *sc* reversal with $y^{8i}$ or $y^{59b}$ was clearly $y^2$ in phenotype. The influence of *sc* upon $y^2$ was similarly demonstrated in triploid females. Females of the genotype $y^{59b}/y^{59b}/y^2$ although lighter in color than wild type were nonetheless distinctly darker than females $y^{59b}/y^{59b}/y^2$*sc*.

Since in Neurospora there is a high correlation between complementation and recombinational (spatial) separation of mutants (CASE and GILES 1958, 1960), it is important to know whether pseudoallelism exists at the yellow locus. Two cases of apparent recombination between noncomplementary *y* mutants have been found. In one, a cross involving attached-X females of the genotype *y sc*

*$w^a ec/y^2 su\text{-}w^a$ $w^{a4} spl$* (*ec*= echinous eye, *$w^{a4}$*= white-apricot[4], *spl*= split bristles) and heterozygous for the autosomal inversions *Cy* (Curly wing) and *$Ubx^{130}$* (Ultrabithorax[130]), a single *$y^+ sc\ w^a ec$* attached-X female occurred. A progeny test established the genotype of this female as *$y^+ sc\ w^a ec/y\ sc\ w^a ec$*. Since *y* has in extensive tests not been observed to back mutate (GREEN 1961), the most satisfactory explanation for the origin of the *$y^+$* female requires two assumptions. First, that *y* lies to the left of *$y^2$*, and second, that a nonreciprocal single crossover occurred between *y* and *$y^2$*. This would lead to the *$y^+$* female of the required genotype. A second *$y^+$* female was found among the progeny of the cross females *$y\ sp^2\text{-}w\ spl/y^{519}\ w^h$*; *$Cy/+$*; *$Ubx^{130}/+$* × males *$y\ w\ spl\ sn^3$* (*$y^{519}$* = a spontaneous *$y^2$*-like mutant kindly supplied by DR. J. SCHULTZ, *$w^h$* = honey-white, *$sp^2\text{-}w$* = spotted[2]-white, a *w* pseudoallele). The exceptional female proved on progeny testing to have the genotype *$y^+\ sp^2\text{-}w\ spl/y\ w\ spl\ sn^3$*. Assuming *$y^{519}$* has its locus, as does *$y^2$*, to the right of *y*, a single crossover between *y* and *$y^{519}$* satisfactorily accounts for the *$y^+\ sp^2\text{-}w\ spl$* chromosome. These results imply the existence of pseudoallelism at the *y* locus.

## DISCUSSION

Although the data do not unequivocally establish pseudoallelism at the *y* locus, they are sufficiently suggestive to warrant the conclusion that complementation need not invariably occur between recombinationally distinct mutants. This conclusion is in keeping with more extensive analyses of complementation in Neurospora (CASE and GILES 1958, 1960). On the other hand complementation between *$y^2$* and *$y^{59b}$* is explicable as complementation between spatially inseparable mutants. Complementation between such mutants is not unique, having been described among certain lozenge mutants (GREEN and GREEN 1956). The derivation of *$y^{59b}$* from *$y^2$* concommitant with a reversion to *$y^+$* is most readily explained as simultaneous mutational events at the *$y^2$* locus. Those detailed arguments submitted by MULLER, CARLSON and SHALET (1961) to rationalize the occurrence of mutations in nondividing chromosomes apply with equal rigor to the concurrent induction of *$y^{59b}$* and *$y^+$* from *$y^2$*. Attempts to explain the occurrence of *$y^{59b}$* as a mutation at a site adjacent to *$y^2$* or as a minute loss proved to be consistently unsatisfactory because they fail to account for the simultaneous occurrence of the reversal event. Other explanations require the occurrence of too many coincidental events to be seriously considered. Because *$y^{8i}$* mutated in the *$sc^{8i}$* chromosome whose left break point is proximal to the *y* locus, a description of its precise spatial relations is precluded.

It is not immediately obvious why *sc* should so strikingly modify the complementation function of *$y^2$* especially since superficially the phenotypes of these mutants appear to be entirely unrelated. Of some interest in this connection is the observation that both *$y^2$* and *sc* together with a number of other seemingly unrelated mutants are partially suppressed by the autosomal suppressor of Hairy wing (*su-Hw*) (BRIDGES 1932; LEWIS 1949). Suppression by *su-Hw* implies that *$y^2$* and *sc* influence a common or related developmental pathway and therefore it is not too difficult to conceive of one modifying the other.

SUMMARY

Complementation at the yellow locus originally described by STONE (1935) is redescribed and extended. Evidence is submitted showing that complementation occurs between allelic mutants. The influence of the mutant scute upon the complementation reaction is demonstrated.

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# X-RAY-INDUCED DEFICIENCIES OF CHROMOSOME 11 IN THE TOMATO[1]

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IDENTIFICATION of the linkage group of chromosome 11 of the tomato by use of the trisomic ratio method has so far been unsuccessful. A trisomic with distinctive phenotype (hereafter designated as 1-134) had been earlier identified cytologically as triplo-11 (RICK *and* BARTON 1954). But other features of 1-134 are anomalous: it had been recovered only once in very large aneuploid progenies of triploids, yet the extra chromosome is transmitted to a relatively high proportion of its progeny; the offspring of 1-134 consistently include triplo-7 and triplo-10. These characteristics suggested that 1-134 might be a tertiary trisomic—a condition also indicated by our recent discovery of rare pentavalents in its PMC's and by trisomic ratios for *cm* encountered in progenies of both 1-134 and triplo-10 (RICK and DEMPSEY, unpublished). Corroboration of the true nature of 1-134 at pachytene would be highly desirable, but such analysis is complicated by problems of a tertiary trisomic and by a condition of general asynapsis characteristic of 1-134.

Since our trisomic collection presumably did not include true triplo-11, the problem of detecting the linkage group corresponding to chromosome 11 was approached by use of X-ray-induced deletions. The most likely candidate was linkage group V, since its tested genes ($a_1$, $f$, $j_1$) have not yielded trisomic ratios with any of the primary trisomics (RICK and BARTON 1954 and unpublished).

## MATERIALS AND METHODS

Pollen of normal nonmutant tomatoes was treated with various doses of X-rays from a G. E. dental unit, filtered with ½ mm of aluminum at 90 kv and an intensity of 300r/min. Immediately after treatment the irradiated pollen was applied to stigmas of a stock of $ms_2$ $a_1$ *hl*, the latter two genes conditioning easily identified seedling characters of group V and $ms_2$, a male sterility that prevents the procreation of any selfed contaminants, which could be highly distracting in such tests. Doses of 5,000, 10,000, and 20,000r were used, but results clearly demonstrated 5,000r to be the most efficient dose.

The immediate progeny of these treatments were grown and searched for individuals with the recessive marker characters. Such individuals were grown

[1] Support for this work from research grant RG-6209 of the U.S. Public Health Service is gratefully acknowledged. We are also indebted to DR. M. M. GREEN for the X-ray treatments.

to maturity for full observation of their phenotypes, tests of fertility, and examination of their chromosomes. For the latter purpose, standard acetocarmine smears were made. Anthers were fixed under aspiration in 3:1 alcoholic acetic and prestained in alcoholic HCl-carmine (LEE 1921) as adapted by DR. S. R. SNOW.

## EXPERIMENTAL RESULTS

This exploratory test proved fruitful beyond expectation. Slightly less than two percent of the progeny of the 5,000r treatment were mutant for one or both markers, and, in terms of reproductive rate, roughly one mutant seedling appeared for every two fruits harvested. This dose evidently had the merits of reducing pollen viability only slightly yet yielding deletions at a workable rate. Classification of the progeny at this dose is summarized in Table 1.

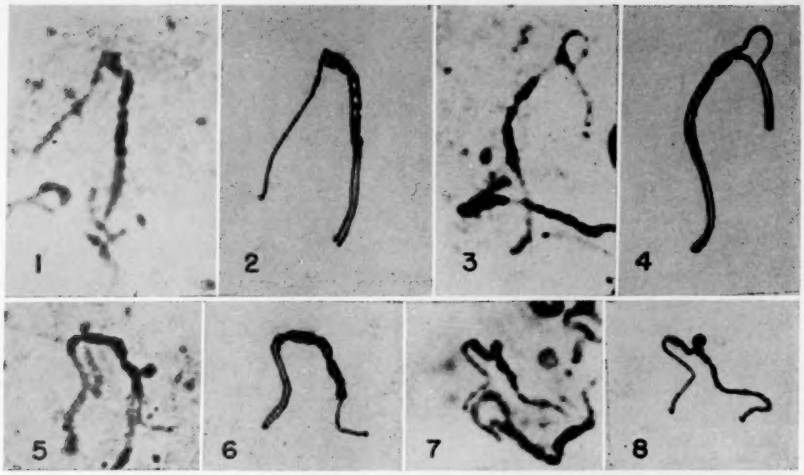
TABLE 1

*Phenotypic classification of progeny from tomato pollen treated with 5,000r*

| | ++ | +*hl* | $a_1$ + | $a_1$ *hl* |
|---|---|---|---|---|
| Number | 2,312 | 19 | 16 | 10 |
| Percentage | 98.1 | 0.8 | 0.7 | 0.4 |

The mutant seedlings, retained and grown to maturity, could be segregated into phenotypic classes for "syndrome" characters of the same nature as those upon which trisomic identifications are based. Representatives of each of these classes were examined cytologically with results that were consistent with the phenotypic identifications. Of the + *hl* group, all except some weak abnormal seedlings conformed to an elongate, erect stature, and relatively vigorous condition. Preparations revealed terminal deficiencies with the achromatic region and most of the chromatic zone of the short arm of chromosome 11 missing for three individuals, and an interstitial deficiency including a short segment of the achromatic region and some of the adjacent chromatic section in the same arm for a fourth plant. Representative deficiencies of both types are illustrated in Figures 1 to 4. The regions missing in the interstitial deficiency could be detected by the loop formed by the normal chromatid (Figures 3 and 4). The plant with the latter aberration displayed more vigor and proved more fruitful than the other three, but otherwise conformed with them in phenotype.

The $a_1$ + plants were remarkably uniform for a broad rugose leaf, short internodes, and considerable vigor. All four cytologically studied plants lacked the achromatic portion of the long arm of one homologue of chromosome 11. In one plant the break appeared to be at the junction of the chromatic and achromatic, two lacked two chromomeres of the chromatic and one lacked one chromomere. A typical example of this type of loss is illustrated in Figures 5 and 6. The majority of the deletions are therefore terminal and all of the breaks occur in or near the proximal chromatic regions. These examinations leave no doubt that group V




FIGURES 1–8.—Tomato pachytene chromosomes showing heterozygous deficiencies for chromosome 11 2,000 ×. Odd-numbered figures are photomicrographs. Even-numbered figures are interpretive camera-lucida drawings. (1 and 2) + *hl* terminal deficiency; (3 and 4) + *hl* interstitial deficiency; (5 and 6) $a_1$ + terminal deficiency; (7 and 8) univalent in haplo-11.

belongs on chromosome 11 with $a_1$ in the achromatic of the long arm and *hl* in either the chromatic or achromatic, but close to the junction between the two in the short arm. Chromosome 11 is therefore the first of the tomato set to have the centromere approximated in its linkage group.

The $a_1$–*hl* plants were, predictably, haploid and monosomic, one of the former and nine of the latter. The phenotypes of these two chromosomal deviants are remarkably well defined. This haploid, like others described for the tomato, had the appearance of a diminutive, dainty diploid. The others conformed uniformly to a weak plant type with few, upright branches, with small, nonpendant leaves having a reduced number of segments, and with flowers tending to be fasciated. The chromosome number 23 was counted in somatic and meiotic plates of three plants in this group, and the univalent was identified as chromosome 11 in pachytene for two plants (Figures 7 and 8). The figures showed no evidence of translocation. Since all the plants of this group conformed in all the aforementioned details to a unique phenotype, including $a_1$ and *hl*, it can be safely assumed that all were haplo-11. These plants represent the first monosomics reported for tomatoes and one of the very few instances for diploid plants in general.

## DISCUSSION

Interest centers particularly upon the production and survival of tomato monosomics. It was heretofore assumed that such tomato aneuploids would be inviable because they failed to appear in the progeny of haploids and asynaptics, whose

disturbed meiosis could be expected to yield N-1 gametes at high frequencies (RICK and BUTLER 1956). The success of our present efforts is most likely vested in averting the elimination in gametogenesis by treating mature pollen. Thus, although asynaptics and haploids yield a high proportion of deficient microspores, none apparently survive gametogenesis. Transmission in matings with haplo-11 might shed light on this problem, but all pollinations thus far attempted have failed to yield progeny. It is interesting in this light that irradiation of pollen also produced monosomics (but of translocation type) in *Petunia hybrida*, whose chromosome number, seven, is the lowest known in the Solanaceae (RICK 1943). Tests are in progress to determine if other tomato monosomics are viable. As with haplo-11, they might logically be sought for the shortest chromosomes of the complement.

Another point of interest concerns the position of the induced breaks. Although the examined sample of eight plants is small, it is doubtless significant that all breaks occurred in or very near the proximal chromatic zones of chromosome 11. A similar high propensity for breakage in this region was found for all tomato chromosomes by GOTTSCHALK (1951) and BARTON (1954). The latter reported a very much higher frequency of breaks in the chromatic zones than in the achromatic following treatment of mature pollen by X-rays and ultraviolet. In tomato material treated premeiotically and examined in pachytene, the former discovered more than twice as many breaks in the chromatic zone but a still higher frequency within the centromere. The short interval between treatment and examination undoubtedly accounts for the much higher survival of centromere breaks in GOTTSCHALK's experiments than in BARTON's or ours. The tomato results contrast sharply with the findings of SAX (1940) in Tradescantia and LONGLEY (1950) in Zea of only slightly higher induced break frequencies near the centromere. The differentiation of tomato chromosomes into chromatic and achromatic regions might account for this striking difference between species.

## SUMMARY

The tomato linkage group V, including the markers $a_1$ and *hl*, was identified with chromosome 11 by means of the radiation-induced deficiency method. Terminal deficiencies were induced in both arms and an interstitial deficiency in one arm of chromosome 11, and plants monosomic for this chromosome were also cytologically identified in the progeny. The gene $a_1$ resides in the achromatic region of the long arm and *hl* in either the achromatic or chromatic close to the junction of the two in the short arm of chromosome 11. The significance of survival of tomato monosomics in this test is discussed.

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# A GENETICALLY CONTROLLED HEAD ABNORMALITY IN DROSOPHILA MELANOGASTER. I. ORIGIN, DESCRIPTION, AND GENETIC ANALYSIS[1]

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MODIFYING genes affecting the expression of inherited characteristics in Drosophila were found almost simultaneously with the first reports of gene mutation in this organism (see review by MORGAN, BRIDGES and STURTEVANT 1925, pp. 40–53). Experiments since this time have shown that modifiers of genetically controlled phenotypic changes are a common phenomenon in hereditary systems (GLASS 1944; GARDNER and STOTT 1951). The first evidence that modifiers existed which would cause the Notch heterozygote to show phenotypic effects other than the typical notch wing pattern was reported by MORGAN (1919). He found modifiers which acted together with the Notch heterozygote to produce changes in wing length, in degree of serration of the notched wing, and in size and shape of the eye. MORGAN was able to select for and localize some of these factors. Others were lost before identification could be made. MOHR (1923) published an analysis showing "Notch" to be a deficiency for a part of the X chromosome. In this account he also discussed a recessive mutant, located on chromosome 3, which he named "enhancer of $N^{g}$."

Several of the existing Notch stocks express in their phenotype modifications other than the typical serrations found on the trailing edge of the wing (HILLMAN 1955, 1956). This paper is a description of the phenomena encountered in these Notch stocks and a study of the genetic interactions influencing the formation of a phenotype exhibiting gross abnormalities of the head. These abnormalities are in some respects similar to those reported by MORGAN in 1919 but which he did not study. The investigation of the genic interactions responsible for these abnormalities and the concepts which can be derived from the study of this particular case represent an attempt toward an understanding of the relationships between the genotype and the phenotype of the adult.

## *Origin and description of the abnormal phenotype*

The mutant phenotype reported here first appeared in a stock culture of $N^{15e}/In(1)$dl-49, *y Hw* $m^{2}$ $g^{4}$. This stock had been kept in the laboratory at room

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temperature in small mass matings from 1945 until 1954. During this period the Notch deficiency behaved in a normal manner, showing the typical notching of the wing, being hemizygous lethal, and having the same effects on embryonic neuroblast differentiation as other Notch deficiencies which had been studied and reported by POULSON (1945). In August, 1954, all stocks in the laboratory were placed in a constant temperature culture room at approximately 18°C. This was an 8° drop from the temperature at which these flies had previously been raised. In the first generation raised at the lower temperature, head abnormalities began to be observed in this Notch stock.

The smallest discernible departure from normal involves a displacement of the ocellar bristles toward the median ocellus. This slight degree of abnormality is difficult to grade accurately because the amount of bristle movement is not constant, varying from a slight median displacement to a drastic change in which the sockets of the ocellar bristles lie adjacent to the median ocellus. The simplest abnormality which can be consistently recognized is a unilateral (Figure 1a) or bilateral fusion of the ocelli, accompanied by a median movement or disappearance of the ocellar bristles. More extreme cases of head abnormality involve a reduction in eye size, formation of an eye stalk, and splitting or duplication of ocelli (Figure 1b,c,d). The most extreme cases also involve a duplication of all or part of the antenna. These are either distal duplications of segments 3–6 (Figure 1e), proximal duplications of segments 1–2 (Figure 1d), or complete duplications (Figure 1f). These abnormalities appear as unilateral or as bilateral expressions (Figure 1b–g). In some of the most extreme cases the eyes become very small, and palps appear in the region between the eye stalk and the antenna (Figure 1h,i).

Upon the recognition of these head abnormalities in the Notch$^{45e}$ stock, a close watch was kept on the other cultures which had been transferred to the lower temperature at the same time. During the succeeding six generations flies with abnormal heads similar to those described above were found in two other stocks: $N^{264-40}$/*In(1)*dl-49, *y Hw m*$^2$ *g*$^4$ and $w^{258-21}$/*In(1)*dl-49, *y Hw m*$^2$ *g*$^4$. These abnormal flies, together with abnormals from the stock which had originally produced the phenotype, were inbred by single-pair matings, and selection for the extreme expression of the abnormal head phenotype was begun.

The viability of these initial inbred lines was extremely poor, and in most cases inbreeding at 18°C led to either loss of the phenotype or extinction of the line. The most consistent abnormalities were found in the $N^{45e}$ inbred stock. Although the extreme expression of the abnormality fluctuated from three percent to 30 percent over 22 generations, brother-sister matings were viable and the abnormality was never lost. All subsequent experiments were performed with this $N^{45e}$ inbred line. It was immediately noted that the head abnormalities were more numerous when the flies were kept at a lower temperature. Development at 18°C resulted in a six to tenfold increase in penetrance and expressivity of ocellar, eye, and antennal aberrations as compared to development at 25°C (Table 1). For this reason, all subsequent experiments for testing the genetic basis of the abnormality were carried through at the lower temperature.

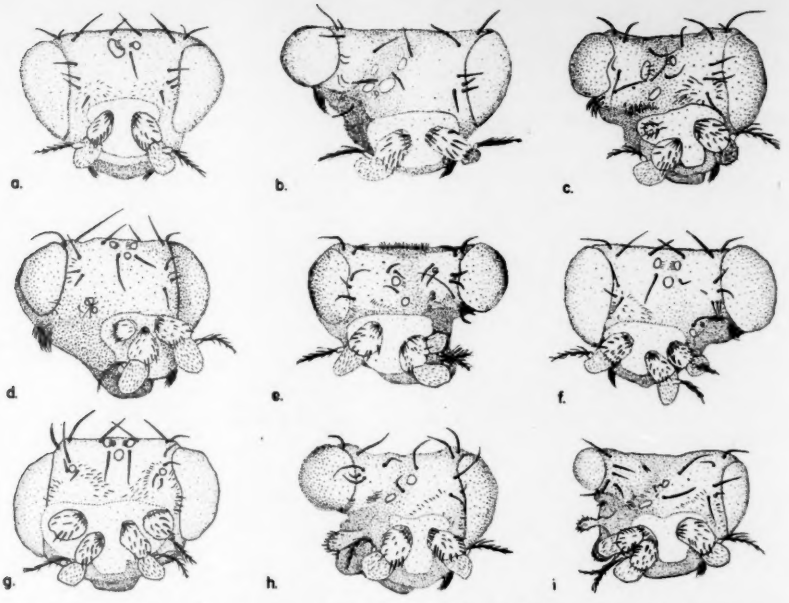


FIGURE 1.—Degrees of expression of head abnormalities which constitute the mutant phenotype.

TABLE 1

*Effect of temperature on penetrance and expressivity of abnormalities*

| Temp. | Total Notch | Total abnormal Freq. | S | Bristle Freq. | S | Ocelli Freq. | S | Eye Freq. | S | Antennae Freq. | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 18°C | 366 | 0.973 | 0.008 | 0.973 | 0.008 | 0.814 | 0.020 | 0.090 | 0.015 | 0.063 | 0.013 |
| 25°C | 326 | 0.546 | 0.028 | 0.546 | 0.028 | 0.083 | 0.015 | 0.015 | 0.007 | 0.012 | 0.006 |

### *Association of the abnormal phenotype with Notch*

The common feature of the three stocks in which the head abnormality appeared is the presence of serrations on the trailing edges of the wings in the heterozygous female. This is the characteristic phenotypic feature of the Notch heterozygote. These three stocks all carry an X chromosome which has either a small deficiency ($N^{45e}$), a nonvisible deficiency inferred on the basis of phenotypic and developmental evidence ($N^{264\text{-}40}$), or a rearrangement ($w^{258\text{-}21}$) in the Notch region. Since no cases of head abnormality have been found in the absence of the wing effect, and since the head abnormalities appeared independently in the three mutant stocks, it has been postulated that changes in the Notch region are primarily responsible for the observed deformation of the head. However, since all of the stocks in which the abnormality had been found were balanced

with an *In(1)*dl-49, *y Hw m² g⁴* chromosome (hereafter designated as the *y Hw* chromosome), it was necessary first to eliminate this inversion X chromosome as a possible carrier of the abnormal head factor.

The *y Hw* balancer from the $N^{45e}$ stock was tested first by breeding an extremely abnormal $N^{45e}/y\ Hw$ female from the inbred line with her *y Hw* brothers. This was repeated several times at both 18°C and 25°C. The results are given in Table 2. At both temperatures used in this experiment the head

TABLE 2

*Brother-sister matings from the inbred* $N^{45e}$ *line.*
$Df(1)N^{45e}/In(1)dl\text{-}49$, y Hw m² g⁴ × $In(1)dl\text{-}49$, y Hw m² g⁴

| Sex | Marker | Head | Number of flies 18°C | Number of flies 25°C |
|---|---|---|---|---|
| ♀♀ | *y Hw* | Normal | 60 | 419 |
| | | Abnormal | 0 | 0 |
| ♀♀ | $N^{45e}$ | Normal | 68 | 325 |
| | | Abnormal | 266 | 55 |
| ♂♂ | *y Hw* | Normal | 474 | 448 |
| | | Abnormal | 0 | 0 |

abnormalities were limited to those females which carried the Notch chromosome. The *y Hw* chromosome in the hemizygous and homozygous states resulted in individuals with completely normal heads. The only question with regard to the results of the experiment is the semilethality found in the *y Hw* females raised at 18°C. This has been shown to be due to causes other than those affecting the formation of the head. That these homozygous females do not carry the factor for head abnormality can be seen from the results at the higher temperature. Here the homozygous *y Hw* females are completely viable and possess normal heads.

To test the balancer chromosome further, it was decided to eliminate it completely and to determine if the abnormalities of the head persisted. A single extremely abnormal female from the inbred line was mated to wild-type males from an inbred Canton-S stock. The results of this cross can be seen in Table 3,

TABLE 3

*A cross of females from the* $N^{45e}$ *inbred line to males from an inbred Canton-S stock*

| Generation | Sex | Genotype | Number of flies Normal | Number of flies Abnormal |
|---|---|---|---|---|
| $F_1$ | ♀♀ | $+/y\ Hw$ | 16 | 0 |
| | ♀♀ | $+/N^{45e}$ | 2 | 4 |
| | ♂♂ | *y Hw/* | 10 | 0 |
| $F_2$ | ♀♀ | $N^{45e}/y\ Hw$ | 97 | 129 |
| | ♀♀ | $+/y\ Hw$ | 282 | 0 |
| | ♂♂ | *+/* | 178 | 0 |

$F_1$. Only flies carrying the Notch chromosome showed abnormal heads. As a further check on the wild-type chromosome, matings were made between the four abnormal females found in this generation and their $F_1$ brothers. The results of the cross show that this wild-type X chromosome had no effect on the phenotype of the head. In this generation, the $+^{4e}/y$ *Hw* heterozygotes were completely normal while the $N^{4se}/y$ *Hw* heterozygotes were over 50 percent abnormal. These experiments support the hypothesis that the inherited factor controlling the abnormal phenotype is located on the X chromosome containing the Notch deficiency.

Evidence for the association of the abnormality with the Notch deficiency itself is more difficult to obtain. This association was tested by crossing extremely abnormal Notch females from the inbred stock to males carrying the markers *sc ec cv* $ct^6$ *v g f*. The $F_1$ Notch females were then mated to their brothers and crossover types were collected from their male progeny. A number of representatives of all single crossover classes between echinus and the centromere were tested. The one of greatest interest here is the X chromosome with the genotype *sc*+*ec*+++++. When the male containing this X chromosome was mated to an extremely abnormal $N^{4se}/y$ *Hw* female at 18°C, the only females showing the head abnormality were those which also had notching of the wings. This indicates that the factor controlling the abnormality, if not identical with the Notch deficiency, at least lies in very close proximity to the Notch deficiency, within 12 crossover units from the distal end of the chromosome.

The evidence cited above supports the hypothesis of a close relationship between the Notch deficiency and the head abnormality. If the relationship is a direct one, it follows that any stock containing a genetic change or rearrangement in the Notch region should be expected to show the phenotypic head abnormality. There was one exception to this expectation among the Notch-deficiency stocks being kept in the laboratory. The cultures of *Df(1)*$N^8$ showed at infrequent intervals a phenotype similar to that described for $N^{4se}$. When, however, abnormal flies were removed from this stock and inbreeding was begun, the abnormality disappeared in the subsequent generation. No line could be isolated from this $N^8$ stock which would consistently show the head abnormality. This type of action paralleled that of "Deformed Eyes" as reported by MORGAN (1919) in the $N^4$ stock.

The behavior of the $N^8$ stock could be attributed to one of two alternatives. Either the deficiency responsible for $N^8$ (a loss of salivary bands 3C1–3D6) has no effect on the formation of the imaginal head, in which case the entire hypothesis involving the relationship between the deficiency and the head abnormality would prove to be fallacious; or there are unknown factors in the genotypic background of the $N^8$ stock which affect the differentiation of the head in such a way that these modifiers combined with the $N^8$ deficiency result in the wild-type phenotype. The second of these alternatives could be tested by reciprocal outcrosses of females and males from the $N^{4se}$ stock to males and females from the $N^8$ stock. If the lack of head abnormalities is due to modifiers in the $N^8$ female, these females outcrossed to males from the $N^{4se}$ stock should show the

abnormal phenotype in their progeny. In addition, offspring of females from the $N^{45e}$ stock, outcrossed to males from the $N^{8}$ stock, should lose the expression of the deficiency on the head within two generations. The tests assume that the modifiers may be carried by the male even though the deficiency cannot be transmitted through the hemizygote. The results of these experiments are shown in Table 4.

TABLE 4

*Reciprocal crosses between the inbred* $N^{45e}$ *line and a laboratory stock of* $N^{8}$ *which did not show the head abnormalities*

(A) $N^{45e}/y\ Hw$ ♀♀ × $y\ Hw$/♂♂ (from $N^{8}$ stock)

| Sex | Marker | Number of flies | |
|---|---|---|---|
| | | Normal | Abnormal |
| ♀♀ | $y\ Hw$ | 2 | 0 |
| ♀♀ | $N^{45e}$ | 20 | 0 |
| ♂♂ | $y\ Hw$ | 14 | 0 |

(B) $N^{8}/y\ Hw$ ♀♀ × $y\ Hw$/♂♂ (from $N^{45e}$ stock)

| Sex | Marker | Number of flies | |
|---|---|---|---|
| | | Normal | Abnormal |
| ♀♀ | $y\ Hw$ | 7 | 0 |
| ♀♀ | $N^{8}$ | 26 | 16 |
| ♂♂ | $y\ Hw$ | 66 | 0 |

The data show that in the $F_1$ of these matings the $N^{45e}$ females had heads which were phenotypically normal, while the heads of the $N^{8}$ females outcrossed to males from this same $N^{45e}$ inbred line exhibited the abnormalities previously discussed. Abnormal $N^{8}$ females from this $F_1$ were again mated to males from the inbred $N^{45e}$ line. In this way a stock of $N^{8}$-abnormal head was inaugurated. This stock consistently showed the abnormal effect. Selection for the abnormality was continued, and after 15 generations of inbreeding by brother-sister matings, 13 percent of the $N^{8}$ progeny showed extreme abnormalities of eyes, ocelli, and antennae. These experiments strongly support the hypothesis that the abnormal head and the notched wing originate in the same genetic change, and they further suggest that the head abnormality is controlled in part by modifiers which are present in the residual genotype of the animal.

### *Modifiers associated with the abnormal head effect*

A series of experiments was designed to test for the presence of modifiers on the X chromosome and on the autosomes of the $N^{45e}$ and $N^{8}$ stocks. It was noted previously that when $N^{45e}$ females were crossed with males carrying the sex-linked mutants *sc ec cv* $ct^{6}$ *v g f*, the Notch females in the $F_1$ generation showed a marked reduction in the penetrance of abnormal head. This phenomenon was utilized in testing for the presence of absence of enhancers of head abnormality

on the X chromosome carrying *Df(1)N*$^{45e}$. Females from the $F_{19}$ of the inbred line were crossed to males of the marker stock. $F_1$ females were backcrossed to males from the inbred line, and the crossover classes of males in the $F_2$ backcross generation were isolated. Individual pair matings were set up for the noncrossover chromosome and for single crossovers involving successive markers from the centromere end, each containing increasingly larger amounts of the original *N*$^{45e}$ chromosome. The results of this experiment are given in Table 5.

TABLE 5

*Test for modifiers affecting head development on the X chromosome containing the* N$^{45e}$ *deficiency*

| Chromosome tested | Total Notch | Frequency of abnormals | |
|---|---|---|---|
| | | Ocellar | Eye |
| *sc ec cv ct*$^6$ *v g f* | 67 | 0.284 ± 0.061 | 0.075 ± 0.053 |
| *sc ec cv ct*$^6$ *v g* + | 171 | 0.585 ± 0.043 | 0.164 ± 0.031 |
| *sc ec cv ct*$^6$ *v* + + | 107 | 0.514 ± 0.053 | 0.131 ± 0.033 |
| *sc ec cv ct*$^6$ + + + | 66 | 0.333 ± 0.063 | 0.045 ± 0.026 |
| *sc ec* + + + + *f* | 20 | 0.100 ± 0.067 | 0.0 |

The original noncrossover chromosome can be taken as the base line for the effect of the marker chromosome on the expressivity of abnormal head. Successive additions of proximal sections of the inbred *N*$^{45e}$ chromosome result in first an increase and then a decrease in expressivity. The initial increase appears first with the addition of the region around and to the right of forked. This remains stable until the region around vermilion is transferred, when the expressivity returns to the base line. These data indicate that there is an enhancer, or enhancers, of the abnormal head effect in the vicinity, or to the right, of forked and a suppressor, or suppressors, of this effect in the vicinity of vermilion on the X chromosome of the inbred line. The double crossover class shown in the last line of Table 5 appears to contain suppressors in both these regions. On the basis of 20 Notch females only a tentative conclusion can be drawn from this double crossover. The evidence, however, supports the conclusion that there are at least two regions on the X chromosome that affect in some way the controlling influence of the Notch deficiency on the development of the abnormal imaginal phenotype.

A second experiment was devised to test for the presence of suppressors of the head abnormality on the balancer X chromosome and the major autosomes of the *N*$^s$ stock. Two matings of the *N*$^s$ stock with a balanced inversion heterozygote stock were made. These matings and the $F_1$ progeny used in further crosses are shown below.

(A) *N*$^s$*/y Hw*, +/+, +/+ ♀♀ × *InS, SM1/Pm, Ubx/Sb* ♂♂
$F_1$ ♂♂ *yHw, SM1/+, Sb/+*

(B) *InS/InS, SM1/Pm, Ubx/Sb* ♀♀ × *yHw*, +/+, +/+ ♂♂
$F_1$ ♂♂ *InS, SM1/+, Sb/+*

The $F_1$ males which were used in further matings were thus autosomal heterozygotes for the marked inversion and for the autosomes from the $N^s$ stock. One set of these had the *y Hw* X chromosome balancer from the $N^s$ stock and the second contained the *InS* balancer from the inversion heterozygote stock. These males, containing known chromosomes, were then crossed in single pair matings to abnormal females from the $N^{45e}$ inbred line. The results are shown in Table 6.

Lines 1 and 2 in this table are the controls; lines 3 through 10 are the experimentals. Line 1 is a sample from the $N^{45e}$ inbred line. Line 2 shows the results of the cross between $N^{45e}$ females and males from the $N^s$ stock. It can be seen that in this heterozygote the penetrance and expressivity of the abnormal phenotype are markedly reduced. Lines 3–6 show the results of crosses between the $N^{45e}$ inbred females and males carrying the *y Hw* X chromosome balancer from the $N^s$ stock. Lines 7–10 show the results of the cross between these females and males carrying the *InS* X chromosome balancer from the inversion heterozygote stock. A comparison of the controls with lines 3 and 7 shows the effect of chromosomes 2 and 3 from the $N^s$ stock; with lines 4 and 8, the effect of chromosome 2; with lines 5 and 9, the effect of chromosome 3; and with lines 6 and 10, the effect of the X chromosome alone. These comparisons clarify the picture of the suppressors present in the $N^s$ stock.

TABLE 6

*Test for suppressors of the abnormal head effect in the* $N^s$ *stock*

| | Genotype | Total Notch | Frequency normals | Frequency ab. ocelli | Frequency ab. eyes |
|---|---|---|---|---|---|
| 1. | $\frac{N^{45e}}{y\ Hw^{(45e)}}, \frac{+^{(45e)}}{+^{(45e)}}, \frac{+^{(45e)}}{+^{(45e)}}$ | 206 | 0.078 ± 0.019 | 0.922 ± 0.019 | 0.029 ± 0.012 |
| 2. | $\frac{N^{45e}}{y\ Hw^{(s)}}, \frac{+^{(45e)}}{+^{(s)}}, \frac{+^{(45e)}}{+^{(s)}}$ | 158 | 0.918 ± 0.022 | 0.082 ± 0.022 | 0.006 ± 0.006 |
| 3. | $\frac{N^{45e}}{y\ Hw^{(s)}}, \frac{+^{(45e)}}{+^{(s)}}, \frac{+^{(45e)}}{+^{(s)}}$ | 114 | 0.965 ± 0.017 | 0.035 ± 0.017 | 0.035 ± 0.017 |
| 4. | $\frac{N^{45e}}{y\ Hw^{(s)}}, \frac{+^{(45e)}}{+^{(s)}}, \frac{+^{(45e)}}{Sb}$ | 90 | 0.856 ± 0.037 | 0.144 ± 0.037 | 0.033 ± 0.019 |
| 5. | $\frac{N^{45e}}{y\ Hw^{(s)}}, \frac{+^{(45e)}}{SM1}, \frac{+^{(45e)}}{+^{(s)}}$ | 111 | 0.541 ± 0.047 | 0.459 ± 0.047 | 0.054 ± 0.021 |
| 6. | $\frac{N^{45e}}{y\ Hw^{(s)}}, \frac{+^{(45e)}}{SM1}, \frac{+^{(45e)}}{Sb}$ | 95 | 0.568 ± 0.051 | 0.432 ± 0.051 | 0.105 ± 0.031 |
| 7. | $\frac{N^{45e}}{InS}, \frac{+^{(45e)}}{+^{(s)}}, \frac{+^{(45e)}}{+^{(s)}}$ | 68 | 0.735 ± 0.053 | 0.265 ± 0.053 | 0.044 ± 0.025 |
| 8. | $\frac{N^{45e}}{InS}, \frac{+^{(45e)}}{+^{(s)}}, \frac{+^{(45e)}}{Sb}$ | 55 | 0.364 ± 0.065 | 0.582 ± 0.066 | 0.164 ± 0.050 |
| 9. | $\frac{N^{45e}}{InS}, \frac{+^{(45e)}}{SM1}, \frac{+^{(45e)}}{+^{(s)}}$ | 58 | 0.207 ± 0.053 | 0.776 ± 0.055 | 0.103 ± 0.040 |
| 10. | $\frac{N^{45e}}{InS}, \frac{+^{(45e)}}{SM1}, \frac{+^{(45e)}}{Sb}$ | 39 | 0.256 ± 0.070 | 0.744 ± 0.070 | 0.282 ± 0.072 |

It can be seen immediately that the X chromosome balancer found in the $N^s$ stock carries a suppressor which drastically reduces the penetrance and expressivity of the abnormal phenotype. With the addition of this chromosome, the number of normals increased by approximately 100 percent while the number of definite abnormals, both of the ocelli and eye, concomitantly decreased. The indication here is that a large measure of the loss of penetrance seen in the cross of $N^{45e}$ females with males from the $N^s$ stock can be attributed to the effects of the suppressor found on this particular *y Hw* chromosome.

Table 6 also shows that suppressor genes can be found on both the second and third chromosomes. A comparison of lines 4 and 5 with lines 3 and 6 indicates that there is a major suppressor on chromosome 2 of the $N^s$ stock and one with a lesser effect on chromosome 3. In this case the suppressor on chromosome 2 reduces the penetrance and expressivity of both the ocellar and eye abnormalities, while that on chromosome 3 reduces only the eye effect. This can mean one of two things. Either (1) the head abnormality is the result of two separate developmental anomalies, one affecting the ocelli, the other affecting the eye, and the action of the suppressor on chromosome 3 is limited to the reactions concerned with the development of the eye; or (2) the development of both the eye and ocellar abnormalities are under the control of the same system of developmental reactions, with the ocellar region being more drastically affected by the disruption due to the Notch deficiency and thus less readily adjusted back to the normal phenotype by the action of this suppressor gene. The second alternative would presuppose that the action of the suppressor on chromosome 3 is not as strong as the action of the suppressor on chromosome 2.

Evidence for the second of these alternatives can be found in the experiment in which the *y Hw* chromosome was replaced by the *InS* balancer before testing for the effects of chromosomes 2 and 3. When the suppressor in the *y Hw* X chromosome is removed, the frequency of ocellar abnormalities follows the same pattern as when this X chromosome is present. However, the frequency of eye abnormalities in the presence of chromosome 2 (line 8) is now intermediate and in the same range as the frequency of eye abnormalities in the presence of chromosome 3. This would indicate first, that these suppressor genes are not autonomous in their action, but that complete suppression is due to the concerted action of all of them working together; and second, that both of these suppressors have an effect on eye and ocellar formation. This, in turn, supports the contention that the eye and ocellar abnormalities are a result of a change in a single developmental process and are not independent events.

We have no evidence to indicate whether the suppressors present in the $N^s$ stock act in an additive or nonadditive fashion. The experiments reported here, although carried out on inbred lines and under controlled temperature conditions still suffer from the effects of environmental variables on penetrance and expressivity. The effects of other variables, such as moisture and nutrition, must be ascertained and controlled before any conclusions can be reached as to the interactions of those suppressors studied here.

*Interrelations between Notch and other cephalic mutations*

Two series of experiments were run dealing with the relationship between the Notch-deficient X chromosome and other inherited factors which affect the development of the head and eyes. These experiments, although not extensive, are of interest and will be presented briefly. The factors used in conjunction with the Notch deficiency may be grouped into two classes; first, those nonallelic to Notch, but with pronounced effects on head development; and second, those which are allelic to Notch, or are located within the limits of the Notch deficiencies.

*Series I:* The first cross made was of inbred $N^{45e}$ females with males from a stock of $sv^{de}/ey^{D}$ which had previously shown duplication of the antennae but which had lost this phenotypic effect. The results are given in Table 7. It is seen that the combination of $N^{45e}$ with eyeless-dominant acts as a lethal in this cross. Whether this lethality is a result of the combination of the deficiency in the X chromosome and the duplication in chromosome 4 or a result of extraneous background genes is at present unknown. A second cross, however, was made between females from an unselected $N^{s}$ stock and males from the above mentioned $ey^{D}$ stock. The results are shown in Table 7. In the $F_1$ generation we can see that the combination of $N^{s}$ and eyeless-dominant is absent. These results support the hypothesis that the absence of the class is due to the presence of the two known genetic factors and opens up an interesting problem concerned with the developmental effects of the combination. Further experimentation is necessary in order to eliminate completely the remainder of the genotypic background, thus verifying the genetic cause of the lethal effect. If the lethality is truly caused by the double heterozygote, a study of the developmental effects of the double heterozygote compared with those of hemizygous Notch and homozygous eyeless-dominant would have a great deal of bearing on the problems of the genic control of head development in Drosophila.

A second cross was made between inbred $N^{45e}$ females and males from a $Cy\ sp^{2}/L^{2}$ stock. The data from the $F_1$ of this cross indicate that there is no lethality associated with the combination of $N^{45e}$ with Lobe or Curly. The interesting phenotypic class in this $F_1$ was the heterozygote $N/+, L^{2}/+$. Although

TABLE 7

*Crosses of Notch stocks with the fourth chromosome mutants* $ey^{D}$ *and* $sv^{de}$.
N/y Hw, +/+ ♀♀ × +/, $ey^{D}/sy^{de}$ ♂♂

| Sex | Markers | Number of flies from female parents | |
|---|---|---|---|
| | | $N^{45e}$ | $N^{s}$ |
| Females | $ey^{D}, Hw$ | 95 | 28 |
| | $ey^{D}, N$ | 0 | 0 |
| | $+, Hw$ | 140 | 90 |
| | $+, N$ | 130 | 69 |
| Males | $ey^{D}, Hw$ | 28 | 12 |
| | $+, Hw$ | 129 | 57 |

there was no lethality shown in this class, the $F_1$ heterozygotes had eyes which appear similar to those found in homozygous Lobe. These eyes were very small and in some cases had the appearance of tiny buttons protruding from the head. It would appear from these results that the combination of the Notch deficiency with Lobe affects the formation of the eye in such a way that fewer facets are formed than is the case with each heterozygote alone.

The above experiments are only of a preliminary nature, but they indicate that developmental reactions exist between Notch and other head mutants. In all of the cases the ocellar and antennal abnormalities observed were limited to flies heterozygous for the Notch deficiency. This can be construed as additional evidence for the association of the abnormality with the absence of the Notch region of the X chromosome.

*Series II:* The second class of experimental matings was between females from the inbred $N^{45e}$ line and males from three separate stocks, each containing a recessive mutant associated with salivary band 3C7. The recessive genes tested were facet, facet-notched, and split. The results from the three experiments are comparable. The $F_1$ generation showed no lethality from the combination of the deficiency and the recessive mutant. In every case the expression of the recessive in combination with the deficiency was greatly increased over that found in the hemizygous or homozygous condition. In the combination of $N^{45e}$ with facet and with split the eye was exceptionally rough. The combination with split caused a larger number of the macrochaetae on the head and thorax to be doubled. When facet-notched and the deficiency were combined, the wings became strap-like with incisions along the median and lateral margins as well as nicks on the trailing ends. In all of these crosses the ocellar and antennal abnormalities were limited to those flies which carried the Notch deficiency. These abnormalities were found with approximately the same frequency as in the inbred $N^{45e}$ line. In the combination, $N^{45e}/fa^n$, a small number of flies were found which had eyes that appeared to be reduced in size and slightly concave. Such an expression is similar to that seen in Deformed and Deformed-recessive, but in this instance it was not as extreme as is found in the case of the latter two mutants. The presence of a deformed phenotype in these flies is in all probability not related to the factor for Deformed located on chromosome 3. Flies showing these concave eyes were infrequent, and when they were outcrossed to males from the inbred $N^{45e}$ line, the eye effects were lost with the breakup of the $N^{45e}/fa^n$ combination. The phenotype was never observed in the inbred $N^{45e}$ or $fa^n$ stocks.

## DISCUSSION

The data presented indicate that the head abnormalities are a result of the action of a number of loci, working together and ultimately culminating in the abnormal phenotype. As far as has been determined, the major genetic factor responsible for the abnormalities is a change in the Notch region of the X chromosome. Other factors present in the residual genotype are dependent upon this change for their phenotypic expression. For this reason the phenotype has

been designated as "Notch-deformed" and will be referred to as such in all further communications. It is necessary, however, to recognize that Notch-deformed cannot be attributed to a change in a single gene. The development of these head abnormalities is under the control of the genotype as a whole. This is a system composed of a major inherited factor whose expression may be modified by enhancers and suppressors located throughout the residual genotype. The terms "suppression," used in the case of the $N^{s}$ stock, and "enhancement," in the case of the $N^{45e}$ stock, refer to the over-all effects of residual genotype on the phenotypic expression of the head abnormalities. The status of individual genes as suppressors or enhancers is based upon the comparison of their effects with known wild-type genes. An effort was made to introduce a completely homozygous wild-type residual genotype into the $N^{45e}$ stock. This, however, proved to be impossible due to the low viability and fertility of flies carrying the $Df(1)N^{45e}$ chromosome together with marked inversions in the rest of the genetic background. Thus, it is not at present known whether a completely wild-type background would enable the major factor to express its effect. Until such an experiment can be carried out, it is impossible to state whether the expression of the Notch deficiency alone is sufficient to induce the head effects and whether the factors in the residual genotypes of the inbred stocks act to increase or decrease this "normal" penetrance and expressivity. Repeated mass matings to an inbred "wild-type" stock have been made, and the penetrance was decreased to approximately 15 percent. This experiment does not, however, answer the question raised above. First, it is not known how much of the X chromosome carrying Notch was replaced by crossing over, and second, it is not known if modifiers of the abnormal effect were present in the "wild-type" stock used. This latter point can be answered only by outcrosses to a large number of inbred lines.

The association in action of a number of genes may be understood in the light of the complex developmental reactions which must take place in the formation of a well organized and integrated structure. The concept that genic interaction is responsible for the normal or mutant phenotype of such a structure is based upon the theory that development is a result of the effect of each of these genes on a particular reaction or series of reactions leading to differentiation of the structure (GOLDSCHMIDT 1938, 1956). If a gene-controlled reaction is quantitatively or qualitatively changed, all succeeding steps dependent upon the product of this reaction will be affected. It must also be remembered that normal development is dependent upon a balance of gene-controlled reactions which have been selected for and stabilized over a long period of time. Small genetic changes may have little effect on the balance when they are taken singly, a greater effect when they are added together. Large changes may be apparent immediately. If GOLDSCHMIDT was correct in his assumption that safety factors exist within the genic control of development, the modifier systems assume an importance in the maintenance and control of these safety factors and of the thresholds which he associates with developmental reactions. One explanation for the results of the experiments reported here is that the Notch deficiency brings about a major

change in the developmental pathways concerned with head formation, but that this major change may be strengthened or obviated by the effects of a series of enhancers or suppressors controlling the developmental reactions associated with the major change.

The large number of modifiers associated with the expression of Notch-deformed can be understood in the above manner. The fact that enhancers of the abnormal head effect made their appearance in the $N^{45e}$ stock is more difficult to explain. Many investigators have noticed that homozygous mutant stocks, mass cultured in the laboratory, have accumulated genes reducing the expression of the mutant characteristic. MARSHALL and MULLER (1917) had attributed this to selection of the more viable wild type over the mutant. SPOFFORD (1956) has shown the accumulation to be due, in part at least, to selection for viability and productivity in the wild type. The Notch stocks reported in this paper had been kept in small mass matings at room temperature for a number of years. Selection was for the penetrance and expressivity of the notched wing. Any modifier which might have arisen on the X chromosome would be perpetuated due to the fact that the deficiency is balanced in an inversion heterozygote. If autosomal modifiers showed some selective advantage at the higher temperature, they could easily become incorporated into the genotype over a period of several hundred generations. Once this incorporation had begun, it would be impossible to dislodge these genes by the accepted laboratory culture methods. Only when the temperature was lowered would their visible phenotypic effects become noticeable. Although hypothetical, since the selective advantage of these modifiers at 25°C has not been tested, this scheme is possible and would explain the results obtained in the experiments reported.

The data indicating the association of a number of modifiers with the appearance of Notch-deformed has a parallel in the studies of erupt (GLASS 1944) and tumorous-head (GARDNER and WOLFF 1949; GARDNER and STOTT 1951). In these cases the phenotypic expression of growths in the eye region is regulated by major autosomal factors while recessive modifiers, on both the autosomes and X chromosome, suppress and enhance the penetrance and expressivity of the abnormality. In both cases the major factor, which is a semidominant gene on chromosome 3, must be present for the appearance of the mutant phenotype. The modifiers by themselves have no effect on the head and ommatidial development. These modifiers, both of erupt and tumorous-head, have been found in a number of "wild-type" stocks of *D. melanogaster*, and modifiers of erupt have been found in *D. simulans* by GLASS (1949). The widespread occurrence of these modifiers of head growths indicates that such genes are not an unusual phenomenon, and the presence in several separate Notch stocks of the modifiers of the Notch-deformed phenotype should not be considered as a rare event. The lack of information concerning the effects of genetic systems such as are reported above can be attributed to the difficulties which one encounters when trying to isolate and study the individual genetic components of the system, independent of the major gene with which they are associated.

SUMMARY

An abnormal phenotype designated as "Notch-deformed" is described. This phenotype is controlled by a series of genes, the major factor being the Notch-deficiency, without which the phenotype is not expressed. Suppressors and enhancers are also concerned with the expression of the phenotype and are found on the X chromosome and the major autosomes. These genetic factors are responsible for head abnormalities involving fusion and splitting of ocelli, reduction of eye size, and duplication of all or part of the antennae. The expression of the mutant phenotype is, in addition, affected by the temperature at which the flies are raised, a low temperature increasing the penetrance and expressivity of the abnormality. The Notch stock containing the mutant genetic background was crossed to stocks containing other inherited factors affecting the development of the head and also to stocks containing recessive factors associated with the region absent in the $N^{55e}$ deficiency. The genetic interactions of these mutants are described, and the general nature of the control of the phenotype by a number of interacting genetic factors is discussed.

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# EFFECTS OF CENTRIFUGAL FORCE UPON CHROMOSOMAL MUTATION IN BARLEY[1]

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INDUCING permanent and self-reproducing changes in living cells provides much inheritable variability for genetic studies. A wide variety of artificial mutagenic agents is known, and it seems to be a useful approach for breeding work. The study of mutation is also a major source of information on the theory of the gene (GOLDSCHMIDT 1955; STADLER 1954).

Experiments were undertaken to find out whether centrifugal force is capable of producing mutations in barley. Phenol, which is known to produce a high mutation rate in various organisms, was used for comparison with centrifugal force.

Centrifugation has often been applied to biological materials, but the problem of mutation has been largely neglected. TANAKA and KAWAGUCHI (1932) and KAWAGUCHI (1936, 1938a,b) obtained triploid and hexaploid silkworms by centrifuging hybrid eggs at 3500 rpm for ten minutes. Some of these were mosaics for the somatic characters studied. KOSTOFF (1937) studied the effect of centrifuging upon the germinated seeds of various plants. They were centrifuged repeatedly for periods of 15–30 minutes at 1000–4000 rpm. Abnormalities obtained included variations in leaf shape, semisterility, and variegated leaves with chlorophyll deficiency. Centrifugation also led to formation of aneuploid and polyploid cells. SAX (1943) found that centrifuged and irradiated Tradescantia microspores had a higher frequency of chromosomal aberration than microspores which were irradiated only. WOLFF and VON BORSTEL (1954) found that preirradiation centrifugation decreased the number of chromosomal aberrations in Tradescantia and Vicia while postirradiation centrifugation increased aberrations. But they were unable to demonstrate any mutagenic influence of centrifugation alone. DODSON and YU (1958) centrifuged barley seeds at 25000*g* for six hours, and found mitotic clumping, extensive fragmentation of chromosomes, and some amitosis in root tip cells. The mutagenic effect of phenol has been tested by HADORN and NIGGLI (1946) and NIGGLI (1948) on Drosophila and by STUBBE

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(1940) on Antirrhinum. AUERBACH (1949) summarized its effects: it produces chromosome breaks in Allium and increases the rate of visible mutations in Antirrhinum.

## MATERIALS AND METHODS

Barley (*Hordeum sativum*) of two varieties was used: Hannchen, which has two seed rows and rough awns, and Montcalm, which has six seed rows and smooth awns. Both varieties are well adapted to Canadian growing conditions. Seeds were germinated on wet filter paper in Petri dishes at room temperature. After 24 hours, they were centrifuged in an International Refrigerated Centrifuge, Model PR-2, operated at 0°C, and fitted with an auxiliary head to permit the use of forces up to 25000*g*. Preliminary experiments were made to test the effect of relative centrifugal force upon germination. The forces used were 2000*g*, 5000*g*, 10000*g*, 20000*g*, and 25000*g* respectively. Seeds were centrifuged for three, six or 20 hours. After centrifugation, the seeds were placed in Petri dishes on wet filter paper at room temperature for germination tests, and the surviving seeds were then grown in the greenhouse in five-inch pots filled with sterilized soil. Because of limited greenhouse space, only 40 seeds were planted for each treatment.

Mitosis and meiosis were studied in root tips and in PMCs, respectively, produced by the surviving plants. For mitosis, the roots were fixed in acetic alcohol (1:3) overnight, hydrolyzed in ten percent hydrochloric acid at 60°C for ten minutes, and then stained with Feulgen reagent. For meiosis, PMCs were studied in ten plants of each treatment. The whole spike was fixed in Carnoy's solution for 24 hours. Anthers were stained with iron-acetocarmine and then smeared.

For a second experiment seeds of the two varieties were centrifuged at 25000*g* for six hours, then planted out in the field. Other seeds were soaked in a 0.06 percent phenol solution for 24 hours, rinsed several times in distilled water, then planted in the field. The combined treatments, either phenol followed by centrifugation or centrifugation followed by phenol, were also used. The various treatments are symbolized as follows: M (Montcalm control), M-RCF (Montcalm-centrifuged), M-P (Montcalm-phenol), M-RCF-P (Montcalm-centrifuged-phenol), M-P-RCF (Montcalm-phenol-centrifuged), H (Hannchen-control), H-RCF (Hannchen-centrifuged), H-P (Hannchen-phenol), H-RCF-P (Hannchen-centrifuged-phenol), and H-P-RCF (Hannchen-phenol-centrifuged). During the winter, 30 seeds of each $T_1$ group (plants grown from the treated seeds are referred to $T_1$, the next generation as $T_2$, etc.) were grown in the greenhouse to produce $T_2$ plants. Cold treatment (0°C for two days) was used to cut down the period of dormancy, so that the seeds harvested from $T_1$ plants could be germinated promptly. Meiosis and phenotypes of both $T_1$ and $T_2$ plants were studied

Hybridizations were also made between the two varieties, as follows: M × H (control), M × H-RCF, M × H-P, M × H-RCF-P, M × H-P-RCF, H × M (control), H × M-RCF, H × M-P, H × M-RCF-P, and H × M-P-RCF. Hybrids were grown in the greenhouse during the winter 1958–1959. Meiosis was studied in the hybrids, and the phenotypes were studied with respect to texture of the awn,

number of seed rows, thickness of the straw, height of the plant, maturation of the plant, fertility of the plant, number of tillers, pigmentation of the leaf, and number of leaves.

## EXPERIMENTAL RESULTS

*Effects of centrifugal force and phenol upon germination:* Tests were made at forces of 2000*g*, 5000*g*, 10000*g*, 20000*g*, and 25000*g*, using 100 Montcalm seeds in each test. The controls had 98 percent germination, while that of treated seed was markedly reduced (Table 1). The percentage of germination decreased as the severity and the duration of treatment increased. Above 20000*g*, most of the seeds were crushed. The lemma and palea were broken. Roots, if present, became transparent, and were often broken off. Such damage, of course, caused the death of the seed. The relationship of percentage of germination to force and duration of treatment was analyzed by the method of linear regression (Figure 1). There

TABLE 1

*Percentage of germination of Montcalm seeds obtained from various centrifuging treatments*

| Treatment | 2000*g* | | | 5000*g* | | | 10000*g* | | | 20000*g* | | | 25000*g* | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Duration (hr) | 3 | 6 | 20 | 3 | 6 | 20 | 3 | 6 | 20 | 3 | 6 | 20 | 3 | 6 | 20 | .. |
| Total seeds | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Dead seeds | 2 | 11 | 25 | 20 | 29 | 46 | 31 | 39 | 62 | 38 | 44 | 78 | 60 | 69 | 94 | 2 |
| Percent survival | 98 | 89 | 75 | 80 | 71 | 54 | 69 | 61 | 38 | 62 | 56 | 22 | 40 | 31 | 6 | 98 |

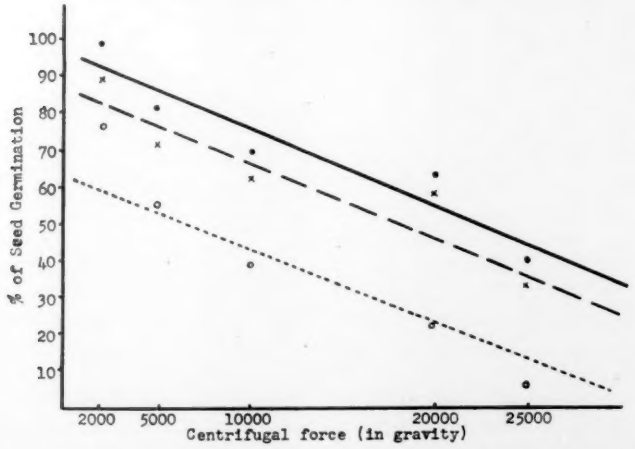



FIGURE 1.—Linear regressions of seed germination. Three-hour centrifugation: Solid line, dots, $Y=94.6-0.002X$, $\bar{x}=12400$, $\bar{y}=69.8$, $b=-0.002$, $t=-5$. Six-hour centrifugation: Broken line, crosses, $Y=86.4-0.002X$, $\bar{x}=12400$, $\bar{y}=61.6$, $b=-0.002$, $t=-5$. Twenty-hour centrifugation: Dotted line, circles, $Y=63.8-0.002X$, $\bar{x}=12400$, $\bar{y}=39$, $b=-0.002$, $t=-5$.

is an approximate inverse linear relationship, with a threshold near 2000*g*, between the force applied and the percentage of germination.

To compare the effects of centrifugal force, phenol, and combined treatments upon germination, centrifugal force at 25000*g* for six hours and 0.06 percent phenol for 24 hours were used. Either treatment alone gave a survival rate of 30 percent. The combined treatments were much more severe, for 11 of 100 seeds survived RCF-P, and ten of 100 survived P-RCF. The mechanism of toxicity of phenol was not investigated, however it penetrates protoplasm rapidly and is known to denature proteins, while the effects of centrifugation are mechanical damage and breakage of chromosomes (see below). Because the two treatments act so differently, one would expect their effects to be additive in regard to direct damage and mutagenic effects.

*Effects of centrifugal force upon root tip cells:* Cytological analysis was made in ten root tips of germinating seeds which had been centrifuged at each of the usual speeds. The force affected the cells in several ways. Some nuclei were shifted to the centrifugal ends of the cells (Figure 2). This was found in ten percent of the elongation cells after 25000*g*, in six percent after 20000*g*, in two percent after 10000 or 5000*g*, and in none after 2000*g*. The majority are not strongly displaced. The treated seeds were allowed to grow over night before slides were made, hence it is probable that many displaced nuclei returned to their normal position before the slides were made. Also, new cells, produced after treatment, were undoubtedly included in the examination.

Centrifugal force may also elongate the nucleus, sometimes to a dumbbell shape, one side often appearing denser than the other (Figure 3). Up to 98 percent of nuclei were elongated after treatment at 25000*g* (Figure 4). Such nuclei were occasionally found after 20000*g* and 10000*g*, but never after milder treatments.

Centrifugal force sometimes interfered with mitosis, for the chromatin clumped, and amitosis occasionally occurred. Amitotic nuclei probably formed by continued distention of the dumbbell shaped nuclei. The principal mitotic abnormality of chromosomes was breakage, which was observed very frequently in the root tips of centrifuged seeds. Breakage was not localized in a particular region of the chromosome. About 30 metaphase cells were found in the whole meristematic region of an average root tip. After 25000 or 20000*g*, about ten percent of the metaphase cells had at least one chromosome showing breakage, sometimes into many fine fragments. At 10000*g* or 5000*g*, one or two metaphase cells of each root tip showed breakage. No breakage was found at 2000*g*. Tetraploid cells were also found at very low frequency, in plants centrifuged at 20000*g* and 25000*g*.

*Effects of centrifugal force upon the pollen mother cells:* Meiosis was studied in PMC's from $T_1$ plants. Fragmentation was the main chromosomal aberration at metaphase I. In some cases, several fragments were completely separated, but sometimes they were connected by a fiber (Figures 5, 6). An interesting chromosomal aberration often observed was the extension of metaphase or late prophase chromosomes into long, slender, *pseudoleptotene* chromosomes. Some chromo-

somes were partly extended and partly condensed (Figures 5, 6). SWANSON, MERZ and COHN (1958) suggested that heterochromatin is generally more condensed and resistant to change, while euchromatin is more pliable and responsive. Thus it seems probable that, in such partially extended chromosomes, the extended parts are euchromatic and the condensed parts heterochromatic. As this was never observed in controls, it is undoubtedly produced by the treatment, and it may well be a specific effect of centrifugation. Bridges (inversions?) were often found at anaphase I (Figure 7), sometimes even two in one cell (Figure 8). Breakage and deletion (Figure 9), might occur at any region of the chromosome interstitial or terminal. Tetrasomic aneuploidy (Figure 10) and translocation (Figure 11) were also found. The relationship of frequency of aberration to the treatments was analyzed by linear regression (Figure 12). While the fit is not close, it suggests linear relationship between acceleration and production of chromosomal aberrations over the range of forces used. However, Table 2 shows that fragmentation (and, to a lesser extent, inversion) occurs over the entire range, while the other effects are rare or absent except after the most severe treatment. Thus, between 20000*g* and 25000*g* there may be a threshold above which extensive disruption of the chromosomes occurs. It is therefore probable that the simple regression line of Figure 12 is actually a compound of at least two different effects.

*Meiotic aberrations in Montcalm and Hannchen $T_1$ and $T_2$ plants:* Meiosis was studied in the $T_1$ and $T_2$ of the eight groups of treated plants, and in the two control groups. Ten plants of each kind were studied. The only aberration found in the controls was fragmentation, which is produced spontaneously under normal conditions at very low frequency. In the treated plants, the main chromosomal aberrations observed in PMCs were fragmentation and pseudoleptotene (Figure 13). Although bridges (inversions?), translocation, chromatid and chromosomal deficiency, breakage, tetrasomic and double tetrasomic aneuploidy (Figure 14), and tetraploidy (Figures 15, 16) were also found, they occurred at low frequency. Translocations were mostly quadrivalents, but hexavalent (Figure 17) and octavalent translocations (Figure 18) were also occasionally found. The results are tabulated in Table 3. $T_2$ plants from the usual eight groups were also studied, with normal Montcalm and Hannchen as controls. $T_2$ plants

TABLE 2

*Meiotic aberrations produced by various centrifugations*

| No. of pollen mother cells | Aberration | 2000*g* | 5000*g* | 10000*g* | 20000*g* | 25000*g* | Total | Percent of totals |
|---|---|---|---|---|---|---|---|---|
| | Fragmentation | 13 | 43 | 37 | 48 | 14 | 155 | 58.0 |
| | Translocation | .. | . | .. | .. | 34 | 34 | 12.7 |
| | Pseudoleptotene | .. | 1 | 1 | 3 | 19 | 24 | 8.9 |
| | Chromosomal deficiency | .. | 1 | .. | .. | 20 | 21 | 7.8 |
| | Inversion | .. | 3 | 2 | 8 | 4 | 17 | 6.4 |
| | Chromosomal breakage | .. | 2 | .. | .. | 12 | 14 | 5.2 |
| | Tetrasomic aneuploidy | .. | .. | .. | .. | 2 | 2 | 0.8 |
| | Totals | 13 | 50 | 40 | 59 | 105 | 267 | |
| | Percent of totals | 4.9 | 18.7 | 14.9 | 22.1 | 39.3 | | |

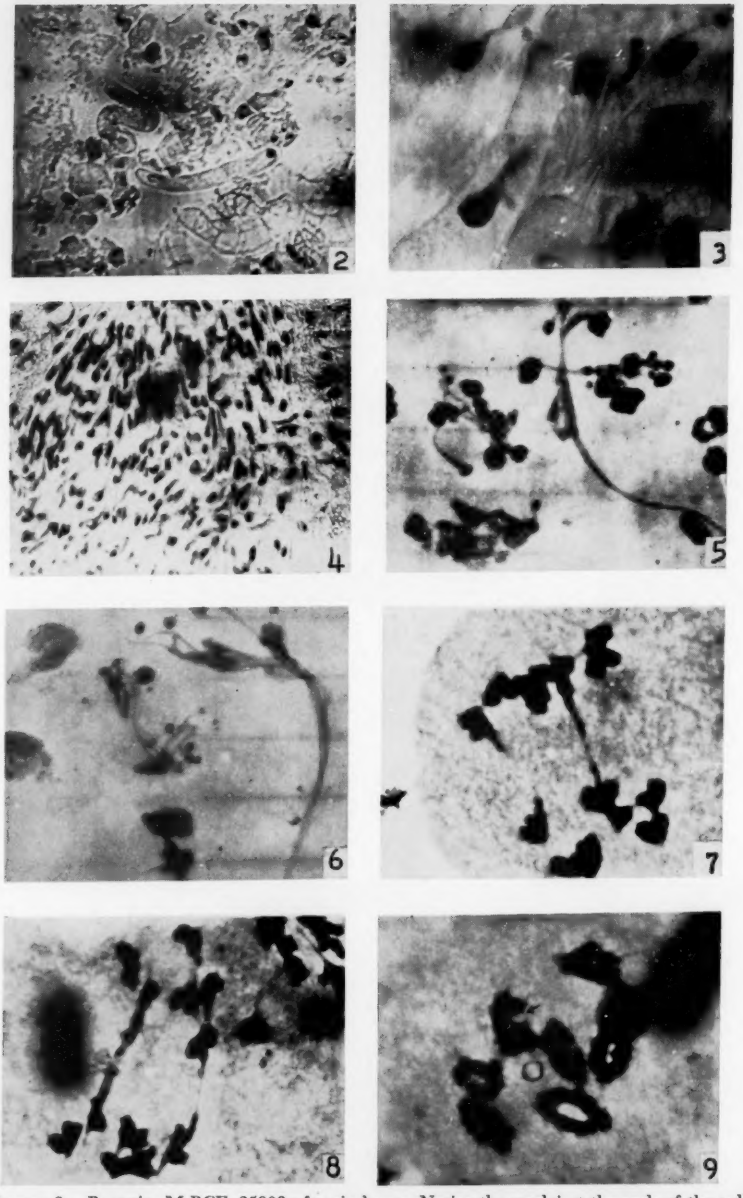



FIGURE 2.—Root tip, M-RCF, 25000*g* for six hours. Notice the nuclei at the ends of the cells. 400×.

FIGURE 3.—Root tip, M-RCF, 25000*g* for six hours. Notice the elongated nuclei. 1000×.

TABLE 3

*Meiotic aberrations observed in various groups of ten $T_1$ plants*

| Aberration | M-RCF | M-P | M-RCF-P | M-P-RCF | H-RCF | H-P | H-RCF-P | H-P-RCF | Totals | Percent of totals |
|---|---|---|---|---|---|---|---|---|---|---|
| Fragmentation | 14 | 23 | 6 | 62* | 160* | 70* | 60* | 100* | 495* | 48.3 |
| Pseudoleptotene | 19 | 3 | 9 | 60* | 65* | 31 | 31 | 79* | 297* | 29.0 |
| Chromosomal deficiency | 20 | 11 | 7 | 0 | 18 | 2 | 8 | 0 | 66 | 6.4 |
| Translocation | 34 | 6 | 5 | 5 | 3 | 0 | 9 | 2 | 64 | 6.3 |
| Chromosomal breakage | 12 | 8 | 0 | 0 | 11 | 3 | 11 | 0 | 45 | 4.4 |
| Inversion | 4 | 4 | 4 | 13 | 4 | 8 | 1 | 2 | 40 | 4.0 |
| Tetraploidy | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 1 | 5 | 0.5 |
| Tetrasomic aneuploidy | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 6 | 0.5 |
| Double tetrasomic | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | 0.2 |
| Totals | 105 | 59 | 33 | 144 | 261 | 114 | 121 | 187 | 1024 | |
| Percent of totals | 10.3 | 5.8 | 3.2 | 14.1 | 25.5 | 11.1 | 11.8 | 18.3 | | |

Number of pollen mother cells

* Count was stopped at this point because of very abundant aberrations.

were grown from seeds taken at random from the spikes of $T_1$ plants, which were semisterile. Only fragmentation was found in the $T_2$ plants, and this was also observed in both controls, though at much lower frequency (Table 4).

*Meiotic aberrations in hybrids:* Ten kinds of hybrids were studied, namely, M × H, M × H-RCF, M × H-P, M × H-RCF-P, M × H-P-RCF, H × M, H × M-RCF, H × M-P, H × M-RCF-P, and H × M-P-RCF. The same kinds of aberrations already discussed were found in these hybrids. The frequencies were low, and the data are summarized in Table 5.

*Phenotypic effects:* Phenotypes of $T_1$ and $T_2$ plants, and of hybrids were examined, with the following results:

1. Seed rows and texture of awn: Hannchen has two seed rows and rough awns, while Montcalm has six seed rows and smooth awns, the Hannchen characters being dominant. One plant derived from the cross of M × H-RCF had six seed rows and rough awns. Again, a plant derived from a similar cross had two seed rows plus an incomplete third row of only three seeds. Another plant derived from the cross of M × H-P-RCF had six seed rows and smooth awns. Two plants derived from the same cross had smooth awns and two seed rows. These effects must depend upon the mutation of the genes of the Hannchen parent to the recessive form, or upon their deletion, thus permitting pseudodominance of the Montcalm genes.

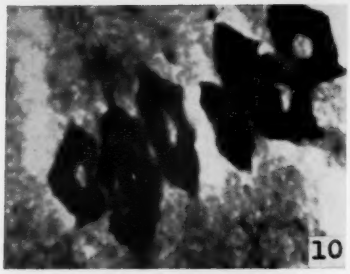
FIGURE 4.—Root tip, M-RCF, 25000*g* for six hours. Notice the elongated nuclei. 400×.

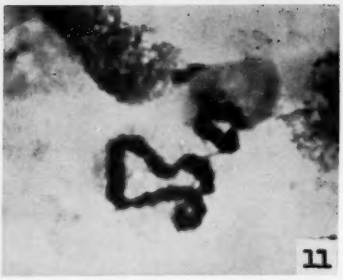
FIGURE 5, 6.—PMCs, M-RCF, 20000*g* for six hours. Pseudoleptotene and fragmentation. 1000×.

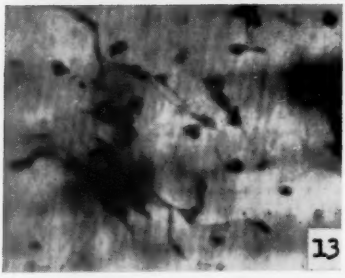
FIGURE 7.—PMCs, M-RCF, 20000*g* for six hours. Inversion bridge at anaphase I. 1000×.

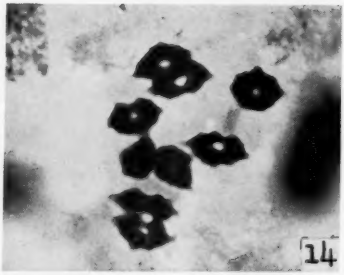
FIGURE 8.—PMCs, M-RCF, 20000*g* for six hours. Two inversion bridges in one cell at anaphase I. 1000×.

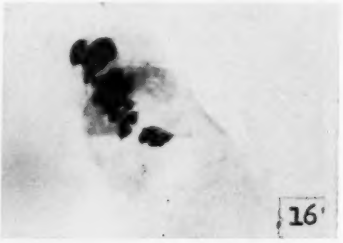
FIGURE 9.—PMCs, M-RCF, 20000*g* for six hours. Arrow indicates chromosomal breakage and deletion. 1000×.





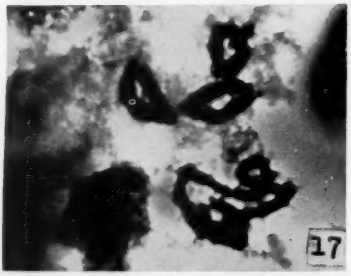



FIGURE 10.—PMC, M-RCF, 25000*g* for six hours. Tetrasomic aneuploidy. 1000×.
FIGURE 11.—PMC, M-RCF, 25000*g* for six hours. Translocation ring. 1000×.
FIGURE 13.—PMC, M-RCF $T_1$ plant. Pseudoleptotene. 1000×.
FIGURE 14.—PMC, M-P-RCF $T_1$ plant. Double tetrasomic aneuploidy. 1000×.
FIGURE 15, 16.—PMC, M-RCF-P $T_1$ plant. Tetroploidy. 1000×.
FIGURE 17.—PMC, M-RCF $T_1$ plant. Translocation-hexavalent. 1000×.
FIGURE 18.—PMC, H-RCF-P $T_1$ plant. Translocation-octavalent. 1000×.

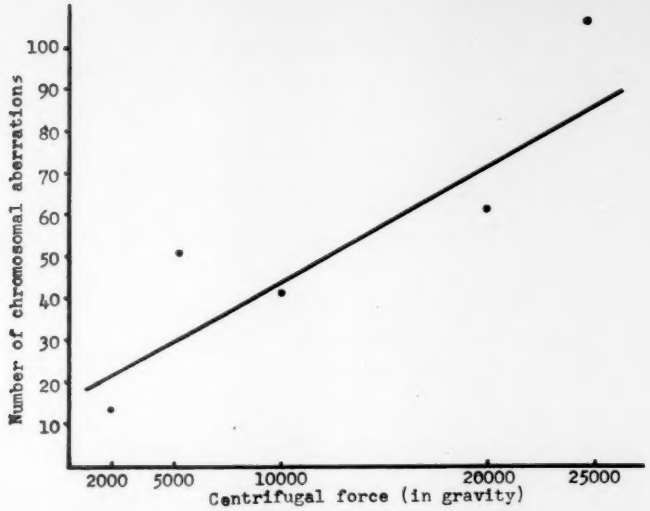



FIGURE 12.—Regression of chromosomal aberration and centrifugal force. Dots: Chromosomal aberrations induced by various centrifugal forces in Montcalm barley. Solid line: Relationship of aberrations to the centrifugal forces. $Y=16.7-0.0026X$, $\bar{x}=9250$, $\bar{y}=40.75$, $b=0.0026$, $t=3.8$, d.f.$=3$.

TABLE 4

*Pollen mother cells showing fragmentation in $T_2$ plants*

| | Treatment | Plant number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of pollen mother cells | M (control) | .. | .. | 2 | .. | 1 | 1 | .. | .. | 3 | .. | 7 |
| | M-RCF | .. | .. | .. | .. | 1 | 1 | .. | 2 | 4 | 5 | 13 |
| | M-P | .. | 3 | .. | .. | .. | .. | .. | 1 | .. | .. | 4 |
| | M-RCF-P | 7 | .. | 2 | .. | 4 | .. | 1 | 6 | 1 | .. | 21 |
| | M-P-RCF | .. | 5 | .. | .. | .. | 8 | .. | .. | .. | .. | 13 |
| | H (control) | 3 | .. | 1 | 1 | .. | .. | 2 | 2 | .. | 1 | 10 |
| | H-RCF | 3 | .. | 2 | 2 | 4 | .. | 1 | 8 | 5 | 3 | 28 |
| | H-P | 2 | .. | 5 | 3 | .. | .. | 1 | .. | .. | .. | 11 |
| | H-RCF-P | 11 | 10 | .. | 9 | 5 | .. | 1 | .. | 1 | .. | 37 |
| | H-P-RCF | 3 | 12 | 4 | 15 | 9 | 8 | 9 | 12 | 4 | .. | 76 |

2. Fertility: partial or complete sterility frequently resulted from the treatments. The partially sterile spike had one to five seeds; the completely sterile spike had no seeds at all (controls averaged 78 seeds for Montcalm, 24 for Hannchen). After treatment, about 15 percent of $T_1$ plants were partially sterile, and about ten percent were completely sterile, the rest being fully fertile. This was not found in $T_2$ and hybrid plants.

TABLE 5

*Meiotic aberrations observed in various hybrids*

| | Aberration | M×H | M×H-RCF | M×H-P | M×H-RCF-P | M×H-P-RCF | H×M | H×M-RCF | H×M-P | H×M-RCF-P | H×M-P-RCF | Totals | Percent of totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of pollen mother cells | Fragmentation | 6 | 64* | 56* | 32 | 97* | 6 | 72 | 117 | 59 | 65 | 574 | 68.8 |
| | Pseudoleptotene | .. | 39* | 15 | 41* | 60 | .. | 6 | 13 | 30 | 31 | 235 | 28.2 |
| | Inversion | 1 | .. | 1 | 5 | 1 | .. | 2 | .. | .. | 1 | 11 | 1.3 |
| | Chromosomal breakage | .. | .. | 8 | .. | .. | .. | . | .. | .. | .. | 8 | 0.9 |
| | Translocation | .. | .. | 2 | 2 | 1 | .. | . | .. | .. | .. | 5 | 0.6 |
| | Tetrasomic | .. | .. | .. | .. | 1 | .. | .. | .. | .. | .. | 1 | 0.1 |
| | Totals | 7 | 103 | 82 | 80 | 160 | 6 | 80 | 130 | 89 | 97 | 834 | |
| | Percent of totals | 0.8 | 12.3 | 8.2 | 8.2 | 19.2 | 0.7 | 8.2 | 15.5 | 10.6 | 11.6 | | |

* Count was stopped at this point because of very abundant aberrations.

3. Maturation of plants: field plantings of treated and control seed were made in May 1958. Controls averaged 90 days maturation time for Montcalm and 100 days for Hannchen. Several M-RCF $T_1$ plants were delayed in maturation for about three weeks, for an average of 110 days. Seeds of $T_1$ plants and controls were sown in the greenhouse on 10 September, 1958. Montcalm controls and $T_2$ plants matured during the second week of January, 1959, about 120 days after planting. Hannchen matured about two weeks later. Eight M-RCF $T_2$ plants, derived from the later maturing $T_1$ plants, ripened in the second week of February, 1959, that is, after about 150 days.

4. Thickness of straw: the late maturing $T_1$ plants of M-RCF had thicker straw than did the controls. The width of the stem of ten $T_1$ and of ten control plants was measured at the second internode below the apex. The controls (M) average 0.42 cm with a variance of 0.00017, while the late maturing $T_1$s averaged 0.60 cm with a variance of 0.00066. Thus the t value is 6.251. With 18 degrees of freedom, the probability is less than 0.001, so the difference is highly significant. The most probable cause is a difference in genotypes caused by the treatment.

5. Height of the plant: One plant derived from a cross of H × M-RCF, was taller than the control, H × M, by almost 90 percent. On the average, the controls of H × M were about two feet high under the greenhouse conditions. This taller plant of H × M-RCF was about 3¾ feet in height. Others were about the same height as the controls.

6. All plants were examined for number of tillers and number and color of leaves, but no significant differences were found.

## DISCUSSION

The purpose of this research is to assess centrifugal force as a mutagenic agent. The data presented show that centrifugal force can produce a great diversity of chromosomal aberrations as well as some phenotypic changes in barley. It is generally agreed that chromosomal rearrangements are an important source of hereditary variability, and, following SINGLETON (1954), chromosomal aberrations are not here differentiated from gene mutations.

An important question is that of whether the genetic effects produced by centrifugal force are similar to those of such mutagens as mustard gas and X-radiation. AUERBACH (1949) compared the effects of the latter two and found a striking similarity. She pointed out that in Drosophila, lethals are scattered along the X chromosome in a similar way by both agents. SMITH (1950) obtained 138 mutants in barley from 2550 seeds (5.4 percent) X-rayed at 16000r. Of these, 16 percent showed a tetravalent ring at meiosis. MOH and NILAN (1956) found eight mutant barley lines (less than 10 percent) induced by the same amount of X-radiation which SMITH used. In the present study, the chromosomal aberrations obtained in the PMCs of Montcalm $T_1$ plants after centrifugation occurred in one to ten percent of the cells examined, and in two to 16 percent of Hannchen $T_1$ cells. Visible mutations, for example, changes in seed rows or texture of awns comprised about three percent. The chromosomal effect resulting from centrifugation is in linear proportion to the dose used (Figure 12) but the above results do not permit a clear decision on whether centrifugal force has mutagenic effects similar to those of mustard gas and X-radiation. Different materials may offer different results, and the same mutagen may have different effects in different organisms, therefore, the same material and method should be used to test this point.

A greater variety and frequency of meiotic aberrations were observed in the PMCs of $T_1$ plants than in those of $T_2$ plants, only fragmentation being found in latter. In the ten samples examined, the total number of PMCs showing fragmentation was seven in Montcalm controls, ten in Hannchen controls, four to 76 in $T_2$ plants (average 28). But in $T_1$ plants, there were from 33 to more than 187 (average 126). The degree of lethality produced by chromosomal aberrations in $T_1$ plants is very high. This may cause the death of the gametes before fertilization, or the zygote may be formed only to die in the early stages of development. A self-pollinating plant with a high frequency of abnormal chromosomes has a high probability of becoming homozygous for any recessive lethal. Barley is a self-pollinating plant, therefore it is not surprising that the sterility of $T_1$ plants was high. The hybrids, however, were produced by a cross of a normal female plant with a treated male plant. The pollen should carry the whole range of mutant chromosomes which were observed in the $T_1$ plants. Yet their hybrid progeny showed only a small proportion of these, both with respect to kinds and numbers. It must be concluded, therefore, that the majority of the aberrations detected in the $T_1$ plants were in fact lethal.

Hybrids were also made for the purpose of determining the effect of centrifugal force on specific gene loci, but the present data are inconclusive. Changes from

two seed rows to six and from rough to smooth awns can be explained either by gene mutations or by pseudodominance based on deletion of these loci from the treated male parent. Reduction of fertility of $T_1$ plants probably resulted from induction of lethal mutants. The late maturing plants with thicker straw might also be based upon mutants of genes influencing these quantitative characters. The same may be said of the exceptionally tall plant. In general, however, further crosses are necessary to establish these changes as valid mutations.

In conclusion, it is possible to state that centrifugal force can be considered as an agent useful in the production of mutations, both chromosomal aberrations and phenotypic changes. It also seems to be a useful new approach, in addition to radiation and chemical mutagens, for production of genetic variation for breeding work.

## SUMMARY

Effects of relative centrifugal force and phenol were studied on two varieties of barley, Montcalm and Hannchen. The percentage of seed germination was markedly influenced by relative centrifugal force, decreasing as the severity of the force and the duration of treatment was increased. Somatic cells of root tips, after centrifugation at a speed of 2000*g* or higher showed that the nuclei might be elongated or shifted to the centrifugal ends of the cells. The chromosomes were broken into fine segments, and tetraploid cells were also found. Numerous chromosomal aberrations were found to occur in the pollen mother cells of the plants derived from centrifuged seed. 0.06 percent phenol for 24 hours seemed to have effects similar to those of centrifugation at 25000*g* for six hours. Changes of the characters of seed row, texture of awn, maturation time and the height of the plant, and the sterility of the seed sets were found after the treatments.

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# RESOLUTION OF GENETIC VARIABILITY IN SELF-POLLINATED SPECIES WITH AN APPLICATION TO THE SOYBEAN[1]

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CONCEPTS for the partitioning of genetic variability for a mating population were developed by FISHER (1918) and have been extended under varying assumptions by numerous authors. Quantification of the nonallelic gene interactions was presented by COCKERHAM (1954) and HORNER, COMSTOCK and ROBINSON (1955). The comments in this paper are modifications of COCKERHAM (1954).

Partitioning of genetic variability involves the identification of a population, such as an $F_2$ population or a randomly mating population, and the definition of a set of genetic parameters relative to this population. Inconsistencies develop when the partitioning concepts are extended to self-pollinated crops: (1) Because of problems in estimation, the partitioning of genetic variability for selfed progeny can be made only with the assumption that the gene frequencies are essentially .5; (2) experimental designs for measuring the variance partitions (if the required number of hybridizations was experimentally feasible) require plots of heterogeneous individuals; however, if competition between rows of soybean genotypes, for example, can create biases in the range of 10 bu/A (HANSON, BRIM and HINSON, 1961), then the interpretation of the yield of a heterogeneous plot certainly would be questioned; and (3) since the natural state for a self-pollinated species is the homozygous condition and selection in most self-pollinated species is normally practiced when the dominance variance is negligible, the definition of the partition of genetic variability in terms of say an $F_2$ distribution leads to a quantification for the genetic variability which has limited utility.

The contribution of this paper is considered original only with respect to the redefinition of concepts as relating specifically to most self-pollinated crops. For a crop such as tobacco which is relatively easy to hybridize, the development would have limited applicability. The concepts will be illustrated with a set of quantitative genetic data from soybeans. An interpretation of the results also will be considered.

[1] Joint contribution from the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and as Journal Paper J-4026 of the Iowa Agricultural and Home Economics Experiment Station, Ames Project No. 1179.

[2] Geneticist, Crops Research Division, Beltsville, Maryland, and Agronomist, Crops Research Division, and Professor, Iowa State University, Ames, Iowa, respectively.

The limiting assumptions made by COCKERHAM (1954), (1) there are two alleles per locus, $A_i a_i$, and (2) the frequencies at one locus are uncorrelated with the frequencies at another locus, $f_{ij} = f_{i.} f_{.j}$, are made in this development. The identification of a base population is essential for two reasons. First, the population serves as the basis for defining a set of genetic parameters, and second, it defines the area of inference for a random sample of observations. The actual state is not an important prerequisite for selecting the base population; rather, the experimental objectivity of the base population is the primary criterion which should be considered. The natural state for the individuals of a self-pollinated species is homozygosity. Problems of plant selection within a population involve conditions where heterozygosity can be ignored. Therefore, a partition of genetic variability which can serve as a meaningful basis for evaluating genetic variability in self-pollinated crops should be made relative to a population of random homozygous lines which an $F_2$ population or an intermated population will generate. The additive portion of the genetic variability would then arise from the average effect of substituting a pair of homozygous alleles into the population.

Consider a population of homozygous lines from a reference population with frequencies of $p_i$ and $q_i$ ($p_i + q_i = 1$) for $A_i$ and $a_i$, respectively. The marginal frequencies for $A_iA_i$ and $a_ia_i$ genotypes in the homozygous lines are $p_i$ and $q_i$, respectively. The description of genetic variability for random homozygous lines follows directly from COCKERHAM (1954). The condition is summarized in Table 1 for the two factor case. The $W_1$ and $W_2$ scales correspond to the marginal effects of homozygotes for the two loci and define the contribution of the loci to the additive genetic variance. With two loci one additional partition is available ($W_3$) which represents the interaction between the additive scales for the two loci. The extension of the concept to $n$ loci (COCKERHAM 1954) yields $\sigma_G^2 = \sigma_{(A)}^2 + \sigma_{(AA)}^2 + \sigma_{(AAA)}^2 + \ldots$ for the genetic variance among random homozygous lines. The parenthesis will be used to differentiate these genetic components from comparable parameters found in the literature. In terms of the two factor case, the contribution of locus 1 to $\sigma_{(A)}^2$ is $p_1q_1(Y_{2\cdot} - Y_{0\cdot})^2$, and the additive by additive component is $(p_1q_1)(p_2q_2)(Y_{22} - Y_{20} - Y_{02} + Y_{00})^2$.

The expected genetic covariance between two homozygous lines arising from

TABLE 1

*Genotypic values, frequencies, and orthogonal scales used to partition genotypic variability of homozygous lines generated by a hybrid population*

| Scale | Genetic type | | | |
|---|---|---|---|---|
| | $A_1A_1A_2A_2$ | $A_1A_1a_2a_2$ | $a_1a_1A_2A_2$ | $a_1a_1a_2a_2$ |
| Yield | $Y_{22}$ | $Y_{20}$ | $Y_{02}$ | $Y_{00}$ |
| Frequency | $f_{22}$ | $f_{20}$ | $f_{02}$ | $f_{00}$ |
| $W_1$ | $q_2$ | $q_1$ | $-p_1$ | $-p_1$ |
| $W_2$ | $q_2$ | $-p_2$ | $q_2$ | $-p_2$ |
| $W_3$ | $q_1q_2$ | $-p_2q_1$ | $-p_1q_2$ | $p_1p_2$ |

a random plant in the $F_n$ generation of selfing is $\rho\sigma_{(A)}^2 + \rho^2\sigma_{(AA)}^2 + \rho^3\sigma_{(AAA)}^2 + \ldots$, where $\rho$ is the expected correlation between the additive scales for the two observations. This expected genetic covariance is identical with the expected genetic variance arising from differences between $F_n$ groups (HORNER and WEBER 1956).

The expected genetic variances for homozygous lines arising from an $F_2$ population are summarized in Table 2. The covariance description used by HORNER and WEBER (1956) is included in the table. For example, $C(3;n,n)$ is the expected genetic covariance between two random lines in the $n$th generation of selfing from a random genotype in the $F_3$. The test generation ($n$) should be sufficiently advanced so that the dominance variation is trivial. The component, $C(3;n,n)$, reflects the genetic differences among groups of homozygous lines arising from random $F_3$ plants and is identical with the expected genetic variance among $F_3$ groups.

Dichotomy in the $F_3$ resulting from the selection of two random $F_3$ plants from each $F_2$ plant is essential for a variance resolution, whereas the additional dichotomy in the $F_4$ contributes little to the variance resolution. A partition of genetic variability based on the complete hierarchical structure is found in Table 3. The group variability involves the variability among group means. If the dichotomy was terminated with any selfing generation, the genetic component for lines within groups will contain all the variability associated with subsequent classes. For example, the variability of lines within $F_2$ groups is $\frac{1}{2}\sigma_{(A)}^2 + \frac{3}{4}\sigma_{(AA)}^2 + \ldots$ while the variability of lines within $F_3$ groups is $\frac{1}{4}\sigma_{(A)}^2 + 7/16\sigma_{(AA)}^2 + \ldots$

TABLE 2

*Partition of genetic variances based on the variability of homozygous lines generated from an $F_2$ population for groups of lines from common sources*

| Source of variability | Description* | Expected genetic variability |
|---|---|---|
| $F_2$ groups | $C(2;n,n)$ | $1/2\sigma_{(A)}^2 + 1/4\sigma_{(AA)}^2 + \ldots$ |
| $F_3$ groups | $C(3;n,n)$ | $3/4\sigma_{(A)}^2 + 9/16\sigma_{(AA)}^2 + \ldots$ |
| $F_4$ groups | $C(4;n,n)$ | $7/8\sigma_{(A)}^2 + 49/64\sigma_{(AA)}^2 + \ldots$ |
| $F_5$ groups | $C(5;n,n)$ | $15/16\sigma_{(A)}^2 + 225/256\sigma_{(AA)}^2 + \ldots$ |
| ⋮ | | |
| Inbreds | $C(n-1;n,n)$ | $\sigma_{(A)}^2 + \sigma_{(AA)}^2 + \ldots$ |

* The test generation ($n$) is assumed to be sufficiently large so that the dominance variance is trivial.

TABLE 3

*Partition of genetic variability of homozygous lines generated from an $F_2$ population based on a hierarchial structure*

| Source of variability | Description | Expected genetic variances |
|---|---|---|
| $F_2$ groups | $C(2;n,n)$ | $1/2\sigma_{(A)}^2 + 1/4\sigma_{(AA)}^2 + \ldots$ |
| $F_3$ within $F_2$ | $C(3;n,n) - C(2;n,n)$ | $1/4\sigma_{(A)}^2 + 5/16\sigma_{(AA)}^2 + \ldots$ |
| $F_4$ within $F_3$ | $C(4;n,n) - C(3;n,n)$ | $1/8\sigma_{(A)}^2 + 13/64\sigma_{(AA)}^2 + \ldots$ |
| $F_5$ within $F_4$ | $C(5;n,n) - C(4;n,n)$ | $1/16\sigma_{(A)}^2 + 29/256\sigma_{(AA)}^2 + \ldots$ |

A pertinent point is the stage of inbreeding required before the genetic tests can be made. In the $F_4$ progeny from individual $F_3$ plants, 7/8 of the loci on the average will be fixed, whereas in the $F_5$, 15/16 on the average will be fixed. Although a genetic analysis could be based on $F_4$ progeny, evaluating before the $F_5$ generation for a critical variance analysis appears questionable for three reasons, the frequency of the heterozygous loci, the necessity of estimating inbred variability from the *n*th generation selfs, and interplant competition.

Techniques for resolving the genetic variability in self-pollinated species would be similar in that they employ single plant progeny rows in an advanced stage of selfing. One procedure would be to use the lines generated from an $F_2$ population. This procedure would utilize the variability structure as outlined in Tables 2 and 3 and would be generated by randomly selecting two plants in the $F_3$ progeny from each $F_2$ plant and advancing the generations of selfing for each $F_3$ line through randomly selected single plant progenies. Recommended selfed generation for testing would be $F_5$. The variability analysis for a single experiment with $p$ $F_2$ groups replicated twice within one environment is given in Table 4. The variability of homozygous lines would be obtained from one randomly selected line for each $F_2$ group. Thus, two estimates would be available from the data for $M_1$. Only $M_1$, $M_2$ and $M_3$ represent independent mean squares. An estimate of the proportion of the total genetic variability associated with epistatic variability is available from the ratio $[3M_2 - M_1 - 2M_3]/[M_1 - M_3]$. This ratio was constructed on the assumption that the principal source of epistatic variability is $\sigma_{(AA)}{}^2$. The estimation procedure is not efficient and would require extensive sampling of the genetic variability.

For simplicity of presentation, the sampling of environments has been ignored but is a necessary feature for such genetic studies. A two plant dichotomy is considered in the $F_3$ generation of selfing. The information for the estimation of $\sigma_{(AA)}{}^2$ is essentially the same for a two or a three plant separation in the $F_3$ when the total number of lines tested is constant. Thus, if the number of $F_2$ groups is not sufficiently large, information can be maintained by sampling three plants in the $F_3$.

Hybridization procedures for self-pollinated species can be developed so that random intermating of an $F_2$ population or of some general population can be

TABLE 4

*Variability analysis for homozygous lines generated by an $F_2$ population with dichotomy in the $F_3$*

| Source of variability | Degrees of freedom | Mean squares | Expected mean squares |
|---|---|---|---|
| Replications | 1 | | |
| $F_2$ group | $p-1$ | $M_1$ | $\sigma_\epsilon{}^2 + 2\sigma_1{}^2 + 4\sigma_2{}^2$ |
| Lines within $F_2$ groups | $p-1$ | $M_2$ | $\sigma_\epsilon{}^2 + 2\sigma_1{}^2$ |
| Error | $2(p-1)$ | $M_3$ | $\sigma_\epsilon{}^2$ |
| Inbreds | | $M_I$ | $\sigma_\epsilon{}^2 + 2\sigma_3{}^2$ |

$\sigma_1{}^2 = 1/2\sigma_{(A)}{}^2 + 3/4\sigma_{(AA)}{}^2 + \cdots$
$\sigma_2{}^2 = 1/2\sigma_{(A)}{}^2 + 1/4\sigma_{(AA)}{}^2 + \cdots$
$\sigma_3{}^2 = \sigma_{(A)}{}^2 + \sigma_{(AA)}{}^2 + \cdots$

accomplished. Within such an intermating population, mating systems can be designed to fit the hybridization problems unique to the crop. In soybeans, for example, pollinating each flower with pollen from different random males produces upon selfing a pattern of relatives as diagrammed in Figure 1. A two-seeded

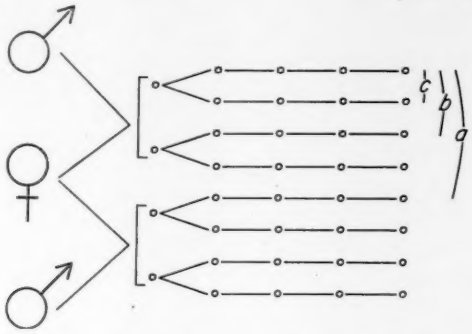


FIGURE 1.—A hybridization plan within a population to obtain the covariances, $C(h;n,n)$, $C(f;n,n)$, and $C(1;n,n)$.

pod gives two "$F_1$" plants from the same parents, whereas seeds in two pods from the same female arose from different males. Two "$F_2$" plants would be advanced from each "$F_1$" plant. Progenies in subsequent generations of selfing originate from a random individual plant within each line. The inclusion of an additional dichotomy corresponding to the "$F_3$" generation at the expense of reduced sampling in the "$F_1$" generation would not be recommended. Gene frequencies need not be .5 for the mating population. Genetic parameters estimated for a population evolved from a representative gene pool would have more meaningful interpretations than those estimated from an $F_2$ population.

In this experimental plan (Figure 1) three covariances and the variability of random homozygous lines with corresponding genetic expectations are available (Table 5). The description of genetic variance involves gene frequencies. The expected genetic covariance for a pair of random homozygous lines ($n$ large) arising from a random "$F_1$", $C(1;n,n)$, is defined without ambiguity. The expected genetic covariance between a pair of lines each arising from one of a pair

TABLE 5

*Partition of genetic variability for homozygous selfed lines obtained from an intermated population where the first generation of inbreeding employed half-sibs and full-sibs*

| Genetic component | | Expected genetic variability |
|---|---|---|
| $C(h;n,n)$ | (a) | $1/8\sigma_{(A)}^2 + 1/64\sigma_{(AA)}^2 + \cdots$ |
| $C(f;n,n)$ | (b) | $1/4\sigma_{(A)}^2 + 1/16\sigma_{(AA)}^2 + \cdots$ |
| $C(1;n,n)$ | (c) | $1/2\sigma_{(A)}^2 + 1/4\sigma_{(AA)}^2 + \cdots$ |
| Inbred lines | | $\sigma_{(A)}^2 + \sigma_{(AA)}^2 + \cdots$ |

of full-sibs is defined as $C(f;n,n)$ and from a random pair of half-sibs as $C(h;n,n)$. Numerous sampling plans could be evolved for this estimation procedure. Each plan would lead to a unique analysis of group variability.

## EXPERIMENTAL DATA

Data available from the study in soybeans reported by HORNER and WEBER (1956) and GATES, WEBER and HORNER (1960) are amenable to an analysis of homozygous line variability. The description of the experimental methods is given in the references. Estimates for the genetic components of variance are given in Table 6 for the attributes, seed yield (bu/A), maturity (days), plant height (inches), seed weight (g/100 beans), percent oil, and lodging score. The study originated with 94 $F_2$ plants. In 1951, two $F_4$ progeny rows (from individual $F_3$ plants) were grown for each original $F_2$ plant. In 1952, two $F_5$ progeny rows were grown from one random $F_3$ plant in each $F_2$ group. This sampling scheme was continued for the $F_6$ and $F_7$ generations. All tests were replicated twice. The analysis for any year is analogous to that given in Table 4; however, the genetic expectations would be modified for each generation; that is, the second year variance sources would involve "$F_3$ groups" and "Lines within $F_3$ groups."

The genetic expectations (Table 7) require ramifications. If one advances an $F_3$ progeny by bulking, the expected genetic variability between $F_2$ groups in the $n$th generation of selfing ($n$ large) is $C(2;n,n)$ provided one can assume that within plot, plant competition is negligible and shifts did not occur in selfing. When testing is done in the $F_4$, the expectations would contain a dominance bias arising from the condition that $\frac{1}{8}$ of the loci on the average are heterozygous. In this study the $F_4$ lines, for example, are considered as $F_4$ lines advanced by bulking to homozygosity. Within plot, plant competition will not be a major factor. Dominance bias ($D_n$) which arises from the condition that $(\frac{1}{2})^{n-1}$ loci are heterozygous on the average is assumed to be negligible. Genetic expectations follow the group analysis outlined in Table 2.

To consider a solution for $\sigma_{(A)}{}^2$ and $\sigma_{(AA)}{}^2$ directly from the eight linear combinations would be questionable since total genetic variability can vary consider-

TABLE 6

*Experimental estimates of genetic components for six soybean attributes (from* HORNER et al., *1956 and* GATES et al., *1960)*

| Year | Component | Yield | Maturity | Height | Seed wt. | Oil | Lodging |
|---|---|---|---|---|---|---|---|
| 1951 | C(2;4,4) | 3.203 | 10.910 | 6.387 | .850 | .224 | .086 |
| | C(3;4,4)—C(2;4,4) | 3.559 | 5.487 | 2.086 | .593 | .118 | .032 |
| 1952 | C(3;5,5) | 9.048 | 19.970 | 5.572 | 1.028 | .253 | .224 |
| | C(4;5,5)—C(3;5,5) | 1.913 | 3.846 | 1.446 | .164 | .126 | .018 |
| 1953 | C(4;6,6) | 9.172 | 17.143 | 17.682 | 1.486 | .343 | .188 |
| | C(5;6,6)—C(4;6,6) | .882 | 2.968 | 1.768 | .235 | .059 | .025 |
| 1954 | C(5;7,7) | 27.009 | 21.802 | 20.753 | 1.483 | .341 | .042 |
| | C(6;7,7)—C(5;7,7) | 1.429 | 2.390 | 1.592 | .058 | .008 | .009 |

TABLE 7

*Genetic expectations for components listed in Table 6 based on a homozygous line analysis and weights used for combining for four sets of estimates*

| Genetic component | Genetic expectations | Combining weight | |
|---|---|---|---|
| | | $\sigma_{(A)}^2$ | $\sigma_{(AA)}^2$ |
| C(2;4,4) | $1/2\sigma_{(A)}^2 + 1/4\sigma_{(AA)}^2 + D_4$* | | |
| C(3;4,4)—C(2;4,4) | $1/4\sigma_{(A)}^2 + 5/16\sigma_{(AA)}^2$ | .054878 | .028125 |
| C(3;5,5) | $3/4\sigma_{(A)}^2 + 9/16\sigma_{(AA)}^2 + D_5$ | | |
| C(4;5,5)—C(3;5,5) | $1/8\sigma_{(A)}^2 + 13/64\sigma_{(AA)}^2$ | .018813 | .011639 |
| C(4;6,6) | $7/8\sigma_{(A)}^2 + 49/64\sigma_{(AA)}^2 + D_6$ | | |
| C(5;6,6)—C(4;6,6) | $1/16\sigma_{(A)}^2 + 29/256\sigma_{(AA)}^2$ | .004387 | .003415 |
| C(5;7,7) | $15/16\sigma_{(A)}^2 + 225/256\sigma_{(AA)}^2 + D_7$ | | |
| C(6;7,7)—C(5;7,7) | $1/32\sigma_{(A)}^2 + 61/1024\sigma_{(AA)}^2$ | .001039 | .000916 |

* $D_n$ represents dominance bias arising from the condition that $(1/2)^{n-1}$ of the loci are heterozygous on the average.

ably between years for all the characters considered. Therefore, estimates for $\sigma_{(A)}^2$ and $\sigma_{(AA)}^2$ were made for the data available from each year. The solution can be considered as a problem in multiple regression where $X_1$ and $X_2$ are the coefficients for $\sigma_{(A)}^2$ and $\sigma_{(AA)}^2$, respectively, $Y$ is the estimated genetic component, and the regression coefficients $b_{y1}$ and $b_{y2}$ are $\hat{\sigma}_{(A)}^2$ and $\hat{\sigma}_{(AA)}^2$, respectively. The information for the separation of the additive and the epistatic genetic variability in the $F_7$ line test is small as compared with the $F_4$ line test. When the solution is considered as a multivariate problem, the information (as the inverse of the variance) is inversely proportional to the diagonal elements of the inverse coefficient matrix. These weights are included in Table 7.

Estimates for $\sigma_{(A)}^2$ and $\sigma_{(AA)}^2$ (Table 8) represent a partition of the within environment genetic variability of homozygous lines generated by an $F_2$ population. The approximate standard errors for the estimates can be determined since each component was expressed as a linear combination of independent mean squares. The standard errors (Table 8) are large and reflect the inadequacies of sampling ($2 \times 94$ lines per test) the genetic variability. Nonsignificant deviation of estimated components from zero reflects the inability to separate the additive

TABLE 8

*Estimates of additive and epistatic components in the partition of the genetic variability for soybean attributes based on a homozygous line analysis with approximate standard errors*

| Measure | Estimated component | | |
|---|---|---|---|
| | $\hat{\sigma}_{(A)}^2$ | $\hat{\sigma}_{(AA)}^2$ | $\hat{\sigma}_{(AA)}^2/\hat{\sigma}_G^2$ |
| Yield | 3.59 ± 7.14 | 8.30 ± 9.50 | .70 |
| Maturity | 19.84 ± 6.38 | 4.80 ± 6.37 | .19 |
| Height | 12.46 ± 3.37 | −1.23 ± 3.33 | < 0 |
| Seed weight | 1.20 ± .57 | .74 ± .60 | .38 |
| Percent oil | .242 ± .128 | .266 ± .130 | .52 |
| Lodging | .242 ± .076 | − .063 ± .088 | < 0 |

and epistatic genetic variability rather than the estimation of total genetic variability.

The significant $\sigma_{(A)}^2$ components for maturity, height, seed weight, percent oil (.10 level), and lodging reflect the plant breeders' experience that these characters are much easier to select for than seed yield. A significant $\sigma_{(AA)}^2$ component for percent oil and the suggestion that epistatic variability could be an important source of genetic variability for seed yield were found in the data.

## DISCUSSION

In general, breeding programs in self-pollinated crops are aimed at selection for a principal character such as seed yield after dominance effects are minimized and yield expression at best approximates the performance of homozygous genotypes. To be interpretable, the resolution of genetic variability in self-pollinated crops should be based on the variability of random homozygous lines from a heterogeneous population. The reference population may be the homozygous lines from a cross or lines which could be generated (for example) by the germ plasm pool of the northern or southern soybean genotypes. This resolution is reasonable since the natural state for a self-pollinating species is homozygosity.

The pertinent question is the continuity between the genetic partitions based on parameters defined relative to an $F_2$ or to a random mating population, when selfed progenies are used in the variability study, and the partitions defined relative to the variability of homozygous lines generated by a population, as developed in this paper. With a homozygous line analysis the additive and additive by additive coefficients are one half and one fourth times, respectively, those when defined relative to the $F_2$ or intermating population. The following justifications can be given for the proposed homozygous analysis: (1) The approach extends the concept for partition of total genetic variability, $\sigma_{(A)}^2 + \sigma_{(AA)}^2 + \ldots$, to the analysis of the genetic variability of selfed progenies and permits a generalization for the partition of genetic variability without restricting gene frequency to .5, (2) the genetic parameters reflect the genetic variability which is characteristic of self-pollinated species, (3) locus contribution has been described in terms of the effect of substituting the homozygous pair of alleles into a population which yields genetic effects and variances which have biological interpretation, and (4) the partition requires the use of advanced single plant progenies which are not subject to within plot, plant competition, a condition which clouds the interpretation of the majority of the quantitative genetic data available to date in self-pollinated crops.

Frequently, in self-pollinated crops the resolution of genetic variability is based on the analysis of $F_3$ progeny rows ($F_2$ groups). The genetic variability can be expressed in terms of the population of random homozygous lines which is $\frac{1}{2}\sigma_{(A)}^2 + \frac{1}{4}\sigma_{(AA)}^2 + \ldots + D_3$, where the dominance bias ($D_3$) results from the condition that $\frac{1}{4}$ of the loci are heterozygous on the average. The assumption is made that plant competition resulting from different genotypes is negligible. Advancing the $F_3$ to homozygosity as bulked $F_3$ lines affects the genetic expecta-

tion only through the dominance bias ($D_3$) which vanishes from the expectation. This result assumes that no genetic shift occurred in selfing. However, a major criticism for the use of $F_3$ progeny data or $F_3$ advanced to the $F_4$ is the biases resulting from the individual plant competition within genetically heterogeneous plots, since the within plot genetic variability is of the order $\frac{1}{2}\sigma_{(A)}^2 + \frac{3}{4}\sigma_{(AA)}^2 + \ldots$ A test based on $F_4$ lines from single $F_3$ plants would certainly have merit. The within plot, plant competition would be reduced. The genetic expectation for random $F_4$ lines from single $F_3$ plants is $\frac{3}{4}\sigma_{(A)}^2 + 9/16\sigma_{(AA)}^2 + \ldots + D_4$ as translated to the variability of random homozygous lines. Dominance bias ($D_4$) results from the condition that $\frac{1}{8}$ of the loci are heterozygous on the average. Again, the expected genetic variance of $F_4$ progeny advanced to homozygosity is identical with the expected genetic covariance between two random inbreds from an $F_3$ plant.

Data selected for analysis required the assumption that the dominance bias could be ignored. Since the 1951 test involved $F_4$ progeny rows, this assumption could be open to criticism; however, in the analyses made by HORNER and WEBER (1956) and GATES *et al.* (1960) for these data and by BRIM and COCKERHAM (1961) for similar type of genetic data, the conclusions were made that dominance could essentially be ignored. A correlation approach was utilized by the authors of these references. Since the variability considered in the correlation approach is that involving the variance component, the coefficients for the linear contrasts are highly correlated. Since reduction concepts due to regression have no genetic interpretation, extreme care must be taken in interpreting these correlations. The primary analysis is the estimate of the genetic parameters based on the linear combination of components defined for the expected genetic variances and is the only analysis considered in this paper.

In the previous analyses of the data, 21 sample covariances and nine variance components were utilized. GATES *et al.* (1960) assumed no epistatic variability and made a linkage analysis, whereas HORNER and WEBER (1956) assumed no linkage and considered only maturity. Total genetic variability differed considerably between environments. In an analysis utilizing 21 covariances and nine variances, the magnitude of the components contributes to the solution. Therefore, only the partition of the within environment genetic variability was considered in this analysis. The partition was determined for each year and the estimates combined based on a weighting proportioned to the information available for each set of data.

The partitions are reasonable. Real $\sigma_{(A)}^2$ components for the measurements other than yield reflect the high heritability normally associated with these characters. If the concepts for protein and oil synthesis as proposed by HANSON, LEFFEL and HOWELL (1961) are acceptable, then epistatic variability should be associated with both percent protein and percent oil. A significant $\sigma_{(AA)}^2$ was found for oil, but protein data were not available. The arguments that epistatic variability is negligible on the genetic scale for a character as complex as yield may be difficult to accept. Since the heritability for yield is relatively low as compared with the other characters considered, an adequate sampling of the genetic variability to establish the partition of the genetic variability will be

difficult. Only a suggestion that epistatic variability could be important for yield was found in the data.

These results differ from those obtained by BRIM and COCKERHAM (1961). Reasons for discrepancies could be inadequacies of sampling for the two studies, the failure to consider the genotype by environment interaction in this study, or the use of bulked, heterogeneous plots in the study reported by BRIM. Only when adequate information on the genetic variability for soybean characters has been accumulated can the final conclusions concerning the partition of genetic variability be made.

## SUMMARY

Homozygosity is the natural state for individuals of a self-pollinated species and is the condition under which selection is normally practiced for the principal quantitative characters. The resolution of genetic variability was made relative to the genetic variability of random homozygous lines which a population would generate. The restriction on gene frequencies was relaxed, and the genetic expectations for covariances between a random pair of homozygous lines from a random plant in the *n*th generation of selfing were developed. The information was used to describe and to discuss experimental designs for measuring epistatic genetic variability in a self-pollinated species and to relate the genetic variability estimated from early selfed generations of testing with the variability of random homozygous lines from a cross.

A partition of the genetic variability for homozygous lines from an $F_2$ population in soybeans was made. Evidence for an epistatic component for percent oil was found. Epistatic variability could be an important source of genetic variability for seed yield, but insufficient information was available to obtain an adequate partition of the genetic variability.

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# INTERALLELIC SELECTION IN EXPERIMENTAL POPULATIONS OF DROSOPHILA MELANOGASTER: WHITE AND SATSUMA

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THE relative adaptive value conferred by a particular allele depends not only on its effects in the individual of specified genetic constitution, on its frequency, its environment, and on population size, but also on interactions with other genotypes (LEWONTIN 1955; PARSONS 1958). Increasing realization of the need to consider together all these factors has led in a number of cases to re-examination of their relative importance in genetic systems which were once considered to have been fully analysed.

MERRELL and UNDERHILL (1956) investigated the course of gene frequency change in moderately isogenic Drosophila populations in which mutant genotypes were allowed to compete, and they concluded that the alleles white ($w$) and satsuma ($w^{sat}$) were apparently comparable to one another in adaptive value. This system has been further examined in the present study.

## MATERIALS AND METHODS

Certain populations were maintained in units of the type designed by REED and REED (1948) and used by a number of later workers, including MERRELL and UNDERHILL. Most of the populations studied, however, have been of larger size, and were kept in cages which permitted total population counts to be readily made (THOMSON 1957, 1961). The entire populations were anaesthetized with carbon dioxide and either counted later from a photographic record, or weighed for estimation of population size from the number in a sample of known mass. At the same time a random sample of adults was taken for determination of gene frequencies and sex ratio; these flies were later returned to the cage.

The course of gene frequency change in this system was also followed in cages which consisted of two communicating sections such that migration of flies from one part to the other was possible but limited (THOMSON 1959). Cages of this type were maintained so that both sections were kept in total darkness, both given full illumination, or one section illuminated while the other was held in darkness.

With the exception of one series of bottle units kept at 20°C, the cages were held at 26°C. Each population was initiated with 20 virgin heterozygous females and ten males of each genotype. Before their introduction into the cages, all flies were aged after emergence for eight days at 20°C.

In initial experiments with isogenic stocks of $w$ and $w^{sat}$ the results differed markedly from those of MERRELL and UNDERHILL, both at 20°C and 26°C. A number of $w$ stocks, all derived from the original $w$ mutant which appeared in T. H. MORGAN's laboratory in 1910, was collected from laboratories in Japan and the United States where they had been separately maintained for many generations. The $w$ stock from Pasadena, the source of MERRELL's stocks (personal communication), differed from the four others tested (THOMSON 1961) in that a significant excess of females was produced under ordinary culture conditions. The second and third chromosomes from the Melbourne and Pasadena lines were introduced into the $w^{sat}$ stock, and the $w$ and $w^{sat}$ strains of each line rendered approximately isogenic by 12 generations of brother-sister single pair backcross matings. In the case of the Melbourne line, a pair of stocks for which this process has been continued for 20 generations was also used, while a third pair of stocks was derived from the original (inbred for 12 generations) by separate mass subculture of each stock for eight generations after the cessation of single pair sib matings.

## RESULTS

In each population studied the frequency of $w$ dropped quite rapidly—a situation different from that observed by MERRELL and UNDERHILL (1956). (Detailed results of the experiments reported here have been placed on file in the Editorial Office of **GENETICS.**) The rate of elimination of $w$ varied with the genetic background of the stocks, so that, for example, 13 weeks after the populations were initiated, the frequency of $w$ averaged 0.15 (calculated from the data for males) for the Pasadena line (three cages) as compared with 0.06 for both Melbourne lines (six cages). The difference in frequency of $w$ in the populations from Melbourne and Pasadena lines in which the gene frequencies were closest after 13 weeks had elapsed was significant (Figure 1, cage E, closed circles, cage NI, open squares; $\chi^2_1 = 5.27$; $P < 0.05$), and these differences were maintained to the conclusion of the experiment after 30 weeks. Results from two cages of each background are shown in Figure 1.

Change of frequency of $w$ in these populations was apparently independent of population size and of temperature within the range 20°–26°C (units Q, R, at 20°C, units M, N, O, P, at 26°C; results on file at the **GENETICS** Editorial Office). With the smaller populations in the REED and REED bottle units, replicates showed somewhat greater variability in the rate of elimination of $w$, but, where the populations were originated from the same stocks, gene frequencies were never as different from cage to cage as were those encountered by MERRELL and UNDERHILL.

Populations held either under conditions of constant illumination or in total darkness showed similar quite rapid changes in the frequency of $w$ (cages J, K; Table 1). When a choice of either dark or light environment (Cages I, II; Table 1) was offered in such cages, the elimination of $w$ followed a pattern significantly different (J compared with I, Table 1: 18 week sample, light and dark sections

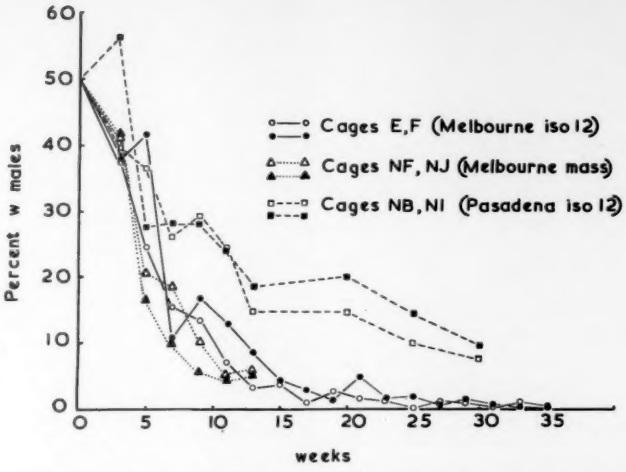


FIGURE 1.—Change in frequency of $w$ in competition with $w^{sat}$ in populations of three different initial genetic backgrounds (results of two replicates shown in each case).

TABLE 1

*Comparison of elimination of* w *from cages held under various environmental conditions (Melbourne iso 12 stocks)*

| Cage | Environment | Percent of $w$ males after indicated number of weeks | | |
|---|---|---|---|---|
| | | 10 | 14 | 18 |
| J | Light | 10.3 ± 2.5 | 4.5 ± 1.4 | 3.3 ± 1.5 |
| K | Dark | 3.3 ± 1.1 | 0.9 ± 0.6 | 2.3 ± 1.0 |
| I | Light | 28.3 ± 5.8 | 17.3 ± 3.1 | 5.1 ± 1.8 |
| | Dark | 29.9 ± 3.8 | 8.1 ± 2.3 | 13.0 ± 2.3 |
| II | Light | 24.6 ± 5.7 | 16.2 ± 4.3 | 11.3 ± 2.6 |
| | Dark | 34.0 ± 6.1 | 20.3 ± 2.4 | 25.6 ± 3.7 |

pooled; $\chi_1^2 = 5.25$; $P < 0.05$) from that observed in populations not subjected to this environmental heterogeneity.

A similar result was obtained for $w$ and its wild-type allele by YANAGISHIMA *et al.* (1953). In the present investigation, the frequency of $w$ was usually higher in the dark section of the cage, but at no time did $w$ tend initially to replace its wild-type allele as in the first of the experiments described by YANAGISHIMA and his colleagues. There may be differential migration of $w^{sat}$ flies to the light side of these multiple environment cages as a result of a more positively phototactic response. It was not possible to continue the experiment for a sufficient length of time to detect an equilibrium between $w$ and $w^{sat}$ if one is reached in such a situation.

The sex ratio in the populations studied varied widely (data on file at the **GENETICS** Editorial Office), without apparent relation to population size or to the frequency of $w$. In certain populations consistent deviations, in either direction from unity, were maintained for long periods. Those populations derived from Pasadena lines (18 samples from three cages) of $w$ and $w^{sat}$ showed a sex ratio which was significantly greater (Deviation/S.E. = 4.01; $P < 0.0001$) than that of the populations derived from the Melbourne lines (iso 20; 18 samples from three cages).

## DISCUSSION

Population experiments, such as those described here, emphasize the contribution made by genetic background to the pattern of frequency change of the marker alleles. This factor is indeed of such importance that it does not seem permissible to specify the adaptive order of a series of alleles except in relation to a particular gene pool. Such a statement has been attempted for the $w$ locus by MERRELL and UNDERHILL (1956). Obviously the experimental populations will diverge genetically over a period of time, but in the present investigation both the frequency of $w$ and the sex ratio were apparently largely dependent on the initial genetic constitution of the stocks utilized in setting up the populations. The increased variability in patterns of gene frequency change in the small populations which they used, together with the difference in genetic composition of their original stocks, probably accounts for the different results obtained by MERRELL and UNDERHILL for the $w$–$w^{sat}$ system.

The factors which might affect the adaptive value of the white and satsuma genotypes in these laboratory populations include relative viability under conditions of extreme crowding in competition with other specified genotypes, differences in development rate, selective mating and other behavioral traits.

Relative viability with respect to that of heterozygous females was estimated under conditions of fairly intense larval crowding; even greater differences might be expected at the higher densities of the population cage food dishes.

Both Melbourne and Pasadena lines showed under crowded culture conditions significantly reduced viability of $w/w$ relative to $w/w^{sat}$ females (offspring of the cross $w/w^{sat} \times w$; Table 2). Any change in intensity of larval competition would be expected to change the result of such tests; neither comparison between lines, nor numerical application of the relative viabilities calculated from these tests to population cage situations, has therefore been attempted. The relative viability of $w^{sat}/w^{sat}$ and $w/w^{sat}$ females did not differ significantly in a similar trial (cross $w/w^{sat} \times w^{sat}$, Table 2) where a progeny test was used to distinguish these two genotypes. No significant differences between the observed heterozygote frequencies and those expected on the basis of the HARDY-WEINBERG distribution were detected in analysis of population samples (data on file at the Editorial Office of **GENETICS**). The viability of $w$ males was consistently a little, although not significantly, higher than that of the $w/w^{sat}$ and $w^{sat}$ genotypes in the Melbourne line cultures.

TABLE 2

*Relative viability of white and satsuma genotypes*

| Cross: $w/w^{sat} \times w$ | Genotype | | | |
|---|---|---|---|---|
| Stock | $w/w$ | $w/w^{sat}$ | $w^{sat}$ | $w$ |
| Melbourne iso 20 | | | | |
| Number of offspring | 464* | 594 | 618 | 654n.s. |
| Relative viability | 0.78 | 1.00 | 1.04 | 1.10 |
| Pasadena | | | | |
| Number of offspring | 379* | 517 | 468 | 492n.s. |
| Relative viability | 0.73 | 1.00 | 0.91 | 0.95 |

| Cross: $w/w^{sat} \times w^{sat}$ | Genotype | | | | |
|---|---|---|---|---|---|
| Stock | $w^{sat}/w^{sat}$ | $w/w^{sat}$ | Females not determined† | $w^{sat}$ | $w$ |
| Pasadena | | | | | |
| Number of offspring | 748n.s. | 744 | 232 | 874 | 868n.s. |
| Relative viability | 1.01 | 1.00 | ... | ... | ... |

* Significant departure from expectation.
† Fewer than four male offspring in progeny test.
n.s. departure from expectation not significant.

No significant differences in the development rate of the various genotypes involved were detected under conditions of extreme larval crowding, despite the considerable lengthening of the larval period resulting from intense competition during that stage.

The fecundity of individual females removed from population food dishes at the time of emergence was extremely variable, as was their body size, even when such females were subsequently kept under optimal conditions. No evidence was obtained of differences in fecundity between white and satsuma genotypes, but it has not been possible to make any estimate of fecundity of females maturing after emergence under cage conditions.

Following the work of MERRELL (1949, and subsequent papers), much attention has been given to selective mating as a factor responsible for the changes in gene frequency observed in laboratory population experiments. REED and REED (1950) used this concept to explain the results of competition between $w$ and its wild-type allele. Good agreement with the gene frequency changes expected on this basis was obtained. Certain later workers (notably MORPURGO and NICOLETTI 1956; NICOLETTI and SOLIMA 1958) cast serious doubt on the validity of direct application of female-choice mating test results to the analysis of population data of this type. These authors, while admitting the occurrence of non random mating, stress the role of differences in larval viability, development rate, fecundity and fertility.

In female-choice mating tests with full illumination (Table 3), $w$ rather than $w^{sat}$ males of the Melbourne iso 12 line were successful in 20.3 percent of cases where a $w/w^{sat}$ female mated with either of two males. When ten (or in a third group of trials, 40) females and ten (or 40) males of each of the two genotypes

TABLE 3

*Results of female-choice mating tests carried out in light and dark (Melbourne iso 12 stocks)*

| Test conditions | Genotype of female | Mating | | | No. progeny |
|---|---|---|---|---|---|
| | | $w$ male | $w^{sat}$ male | Indeterminate* | |
| Light | $w^{sat}/w^{sat}$ | 11 | 76 | 0 | 2 |
| | $w/w^{sat}$ | 77 | 419 | 22 | 48 |
| | $w/w$ | 18 | 74 | 1 | 7 |
| Dark | $w^{sat}/w^{sat}$ | 43 | 57 | 3 | 7 |
| | $w/w^{sat}$ | 125 | 157 | 14 | 60 |
| | $w/w$ | 38 | 39 | 0 | 9 |

* Fewer than four males emerged from culture.

were present in the same mating chamber, only 7.0 percent of the successful males were of the $w$ constitution. Similar results were obtained with flies from the Pasadena lines. The success of $w$ in single female-two male as compared with multiple tests is probably due to masking of the individual variation of sexual activity of the $w^{sat}$ males by the use of a number of them. Both white and satsuma females showed the same preference for $w^{sat}$ males, regardless of the length of time for which they were aged before being mated, a situation different from that encountered by RENDEL (1951) who found that vestigial males performed better in competition with ebony males when both were young.

When the mating tests were carried out in total darkness, the $w^{sat}$ males were still significantly favored by $w/w^{sat}$ females (55.1 percent of successful matings, $\chi^2_1 = 4.8$, $P < 0.05$; Table 3), but there was a very highly significant difference ($\chi^2_1 = 116.5$, $P \ll 0.001$) between the results of female-choice experiments carried out in full illumination and in total darkness. Dependence of the apparent intensity of selective mating on conditions of illumination has also been observed in a number of other instances (e.g. RENDEL 1951). The rate of change in frequency of $w$ from the cage populations was independent of illumination unless the environment was in this respect heterogeneous, although it was markedly different in populations derived from isogenic stocks (Melbourne and Pasadena) of different genetic background, despite the apparent identity in female-choice tests of mating preference of females from the two stocks. For these reasons it would appear that selective mating cannot be, by itself, the principal determinant of gene frequency in this system.

HARLAND and JACKSON (1958) and SAKAI, NARISE, HIRAIZUMI and IYAMA (1958) have demonstrated that differences in activity exist between various genotypes. It is possible that behavioral differences of this type might contribute to the determination of the relative fitness of a given genotype under certain environmental conditions. The former authors have not in a strict genetical sense shown that white is at an "advantage" with respect to wild type in a particular environment, for such a conclusion must be based on a knowledge of the relative contributions, made by each of the genotypes involved, to the gene pool of the succeeding generation.

Using a modified form of the apparatus of HARLAND and JACKSON, comparison of white and satsuma genotypes revealed highly significant differences ($\chi_1^2 = 16.07$; $P < 0.001$) in the ability of males compared with females to reach food surfaces within a test period of 24 hours. In these experiments, carried out in total darkness, the flies were introduced into an empty central chamber to which two other chambers were connected by narrow tubes. These compartments contained cellulose tissue soaked in one case with water, in the other with yeast suspension. A distinction could thus be made between response to a humidity gradient and a response to the stimulus provided by food. While $w^{sat}$ males tended to show a uniform distribution throughout the test apparatus, $w$ males congregated about food surfaces (Table 4) which they reached more quickly than did the $w^{sat}$ males. If there is a tendency for mating to occur at the food surfaces, as in otherwise widely dispersed natural populations of Drosophila, $w$ might be favored. In laboratory populations such differences, unless they reflect variation in the general activity of the flies, are likely to play a relatively minor part in the determination of gene frequency.

TABLE 4

*Behavior patterns of white and satsuma genotypes based on combined data of 41 tests (Melbourne iso 12 stocks)*

| Genotype | Food surface | Central chamber | Moist surface |
|---|---|---|---|
| $w$ | 319 | 210 | 77 |
| | (52.6)* | (34.7) | (12.7) |
| $w/w$ | 135 | 35 | 19 |
| | (71.4) | (18.5) | (10.1) |
| $w^{sat}$ | 152 | 168 | 64 |
| | (39.6) | (43.7) | (16.7) |
| $w/w^{sat}$ | 328 | 119 | 44 |
| | (66.8) | (24.2) | (9.0) |

* Figures in parentheses are percentages of genotypes in the chambers at the completion of the test.

SUMMARY

The rate of elimination of $w$ and spread of $w^{sat}$ in laboratory populations is largely independent of population size, but depends chiefly on features of the genetic and environmental backgrounds. It is suggested that selective mating plays a minor part in the determination of gene frequencies in this system, while the reduced larval viability of the $w/w$ genotype under conditions of intense larval competition may be important. Sex ratio is an intrinsic characteristic of each population and varies with the genetic composition of the stocks employed, but is independent of population size and the frequency of $w$ and $w^{sat}$.

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# THE INHERITANCE OF SELF-INCOMPATIBILITY IN HYBRIDS OF LYCOPERSICON ESCULENTUM MILL. × L. CHILENSE DUN.[1,2]

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BOTH self-fertile and self-incompatible species are found in the genus Lycopersicon (LAMM 1950). The self-incompatibility in *L. peruvianum* was shown to be of the Nicotiana or multiple-allelic gametophytic type by McGUIRE and RICK (1954). Less extensive evidence suggested similar genetic control in *L. hirsutum*, *L. glandulosum*, and *L. chilense* (*L. peruvianum* f. *dentatum*). In addition, the inheritance of self-incompatibility in $F_1$ hybrids of *L. esculentum* × *L. peruvianum* was investigated. All the plants of both generations were self-incompatible. The $F_1$ hybrids from a given cross were of two intraincompatible, intercompatible types determined by the incompatibility (*S*) allele inherited from *L. peruvianum*. The pollination relationships of the $F_2$ indicated a recombination of *S* alleles, as expected, and also a sporophytically determined restriction of crosses of the $F_2$ to unrelated pistillate *L. peruvianum* plants. This barrier was interpreted as the action of one or two genes from *L. esculentum*.

LEWIS and CROWE (1958) have summarized the results of many attempted hybridizations of self-fertile with self-incompatible species. Usually such crosses are successful only when the self-fertile species is used as female. This phenomenon was termed unilateral hybridization by HARRISON and DARBY (1955). The exceptional self-fertile species which can successfully fertilize self-incompatible types are suggested by LEWIS and CROWE (1958) to be intermediates in the evolution of self-fertility from self-incompatibility.

In only one case has the inheritance of self-incompatibility been studied in such crosses beyond the $F_2$ generation. In hybrids of *Petunia axillaris* × *P. violacea*, MATHER (1943) found a dominance of self-incompatibility but a weakening of the reaction in the backcross hybrids. He attributed this to the dilution through backcrossing to the self-fertile parent of the polygenes affecting self-incompatibility.

In the present study the genetic differences between *Lycopersicon esculentum* and *L. chilense* with respect to self-incompatibility and related morphological characteristics were investigated in $F_1$, $F_2$, and backcross generations.

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[2] Part of a thesis submitted to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Genetics.

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## MATERIALS AND METHODS

All materials were supplied from the collections of Dr. C. M. RICK, University of California, Davis, California.

*L. chilense* was represented by six collections made in southwestern Peru between 1938 and 1957. All were self-incompatible. A single plant derived from a collection made in Tacna, Peru, in 1942 by GOODSPEED was used as the parent of hybrids with *L. esculentum* and as one source of inbreds (Figure 1). Other collections were used to verify the type of self-incompatibility and to test the crossability and level of fertility of the hybrids. All collections showed the typical features of *L. chilense* (RICK and LAMM 1955): erect habit, brittle stems, pinnately divided leaf with many segments, very long peduncles, large and showy yellow flowers, exserted styles, and green berries.

As a standard recurrent parent representing the self-fertile species, *L. esculentum*, the variety Tiny Tim was used (Figure 1). This variety originated as a selection from the cross Window Box × Red Currant (*L. pimpinellifolium*). Its small size, abundant fruit and adaptability to growth in small pots in the greenhouse make it an excellent experimental material. In contrast to *L. chilense*, this tomato has determinate plant growth, stubby, inconspicuous flowers with included styles, and red fruit.

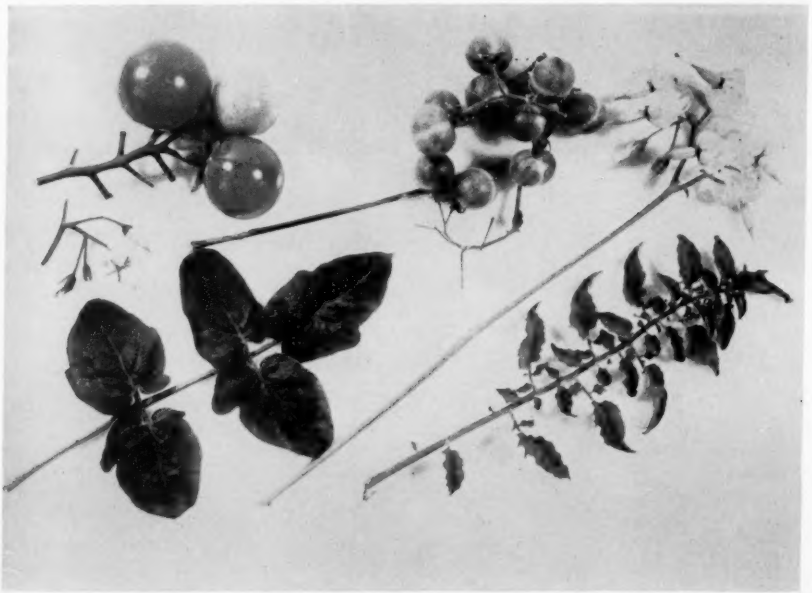

FIGURE 1.—Leaf, flower cluster, and fruit of *L. chilense* (right) and *L. esculentum* var. Tiny Tim (left).

Two *L. esculentum* stocks carrying marker genes, and the Pearson variety, a large-fruited commercial type tomato, were also used as recurrent parents for some backcrossing experiments.

The $F_1$ hybrids were obtained by embryo culture by Dr. C. M. Rick. Pearson was the female parent. Two hybrids were used representing the two possible intraincompatible, intercompatible types (McGuire and Rick 1954) determined by the two *S* genes of a single *L. chilense* parent. The plant identified herein as 4-2 carried $S_1$, and that identified as 4-8 carried $S_2$. The $F_1$ hybrids were self-incompatible. Their crossing relationships as determined by Rick (unpublished) and verified here are given in Figure 2.

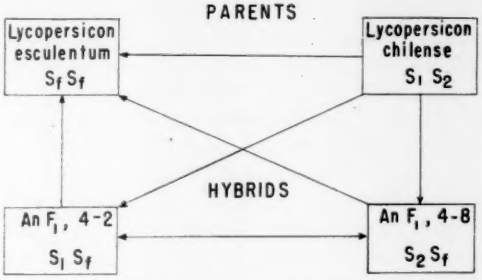


Figure 2.—Crossing relationships of *L. chilense* and *L. esculentum*. Arrows point from pollen to pistillate parent for the successful crosses.

All backcrosses were made using the self-fertile parent as female and the self-incompatible as male. The experimental materials were all grown in fumigated soil, in pots in an insect-free greenhouse maintained at 75°F during the day and 65°F during the night. The *L. chilense* parent plant and the $F_1$ hybrids were maintained clonally whereas the remainder of the materials were grown from fermentation-cleaned seed.

Buds were pollinated after careful emasculation to avoid contamination of pollen. Pollinated flowers were tagged or marked appropriately. Self-incompatible plants were pollinated at anthesis without emasculation. Pollen was often stored until use at 32°F under dry conditions.

Self-incompatibility was scored by the evaluation of a group of tests, supplemented, when necessary, by experienced judgment. The first five or more flowers of young plants were self-pollinated at anthesis and tagged, whereupon the plants were set aside for observation. Self-fertile plants normally set fruit quickly and pea-sized fruit developed in seven to ten days. When two or more fruit developed on a plant, the plant was considered to be self-fertile. Decisive scoring of self-incompatibility was more difficult, for unfruitfulness was often due to poor fertility (see results). Such plants were therefore further pollinated. Those that set fruit with *L. chilense* and with the $F_1$ hybrid carrying the other *S* gene, but failed to set fruit when pollinated by *L. esculentum* were considered self-incom-

patible. When a question of self-fertility still existed, pollinated styles were examined for pollen tube growth and the incompatibility reaction. Only about two percent of all plants tested were unclassifiable, and these were weak or sick.

Pollen tubes were observed in fixed styles under ultraviolet light using the method of MARTIN (1959). Pollen tubes fluoresced bright yellow and stoppage of pollen tube growth was clearly evident after incompatible pollination. Pollen tube growth was measured using a calibrated objective with a binocular dissecting microscope by measuring from the surface of the stigma to that point where an estimated 90 percent of the tubes were inhibited.

Inbreds of *L. chilense*, desired for further study of self-incompatibility in the parent plant, were obtained by treatment of individual flowers after self-pollination with one percent alpha naphthalene acetamide in pure lanolin smeared on the anther column, corolla or calyx (EMSWELLER and STUART 1948). The treatment resulted in regular fruit set, and seed set at the mean rate of 0.16 seed per pollination. In comparison, thousands of self-pollinations without hormone treatment failed to set. Compatible crosses yield 50 or more seeds per pollination.

Certain characteristics of the mature flower were measured to see if any of the differences between *L. esculentum* and *L. chilense* were associated with self-incompatibility and were therefore carried along during the backcrossing process. In the parent materials including Pearson, the $F_1$, $F_2$, and a sample of the backcross hybrids, measurements were made of the length of style, length and width of corolla segments, length and width of anther column and length of peduncle. Counts were made of the number of flowers per cluster.

Marker genes were scored in all families in which they segregated.

The number of classes of offspring with respect to incompatibility occurring in a single $F_1$ family of *L. chilense* plants was determined by a screening technique (MCGUIRE and RICK 1954). A given plant was chosen as pollen parent and all other plants were pollinated by it. A standard pollination consisted of two clusters of three flowers each. The plants which failed to set fruit were concluded to belong to the same group as the pollen parent. The fruitful plants consisted of several groups which were further screened out by the choice of other pollen parents and further rounds of pollinations. Intragroup cross-fertility was tested by pollinating five plants of each group in all possible combinations.

## RESULTS

*Inheritance in* L. chilense *and hybrids with* L. esculentum: The evidence collected from a number of sources leaves no doubt that the self-incompatibility in *L. chilense* is of the Nicotiana or multiple-allelic gametophytic type. In each of two reciprocal $F_1$ families of *L. chilense*, four intraincompatible, intercompatible groups were found. The actual numbers were 11, 12, 7, and 5 in one family and 9, 12, 8, and 11 in the corresponding classes of the other family. The segregation of the 75 plants tested was in a 1:1:1:1 ratio ($\chi^2 = 3.456$). One anomalous plant not fitting into one of the four groups above appeared to result from a stray pollination. The four groups of one family corresponded to the four groups of

the other. No reciprocal differences in compatibility were found. In the inbred generations, plants were found of the constitutions $S_1S_1$, $S_1S_2$, and $S_2S_2$, but too few inbreds were obtained to test for ratio. The compatibility relations of these plants were in accordance with the Nicotiana scheme.

Further evidence for the multiple-allelic hypothesis was obtained from the hybrids of *L. esculentum* × *L. chilense*. Only two incompatibility classes, determined by the two *S* alleles present, were found in the $F_1$ by RICK (unpublished). The segregations found in $F_2$ families were skewed in the direction of the *L. chilense* type (i.e., in one family there were 32 $S_1S_2$ to 18 $S_2S_f$). Two factors were probably responsible for this deviation. First, no recombination for the self-fertility allele of the *S* series might have occurred due to an inhibition of pollen tubes carrying $S_f$ by all self-incompatible plants. Second, a high degree of pollen and seed sterility could have offered opportunity for selection.

Apparently because of selection against pollen containing the self-fertility allele, all $F_2$ plants contained at least one *S* gene for self-incompatibility. However, a weak self-fertility in some plants was manifest in a segregation for degree of pollen tube inhibition, in full pollen tube growth in some cases, and in very limited fruit and seed set. These results contrast with those of MCGUIRE and RICK (1954) in $F_2$ hybrids of *L. esculentum* × *L. peruvianum* where no such attenuated self-incompatibility was found.

Five small families were grown which, with respect to *S* alleles, comprised $F_2$ generations. These were obtained by crossing self-incompatible plants of the third backcross generation (see later) to an $F_1$ plant carrying the other *S* allele. The plants were expected to be of two genotypes, $S_1S_2$ and $S_2S_f$. All of the families were similar in that each contained both self-fertile and self-incompatible progeny. Among a total of 87 plants, 39 were classified self-fertile and 48 were self-incompatible. The results agree with those of the true $F_2$ except that the self-incompatibility was weaker, perhaps due to the loss through backcrossing of some polygenes. The styles of the incompatible plants revealed that segregation for degree of pollen tube inhibition was extreme. The self-fertile plants probably represent one tail of the segregation. The genotypes of these plants with respect to *S* alleles was not determined.

Crosses of the $F_1$, $F_2$, and backcross hybrids to unrelated, pistillate *L. chilense* plants were seldom successful. Observations of the style showed poor germination of the pollen, but uninhibited pollen tube growth. Successful crosses were characterized by various degrees of embryo abortion. Taken together, the observations point to a multigenic influence of *L. esculentum*.

*Backcross transfer of self-incompatibility*. The $S_1$ allele was transferred to *L. esculentum* (Tiny Tim) through six backcrosses and the $S_2$ was transferred through three. Furthermore, $S_1$ was transferred from incompatible plants of the second backcross generation to two *L. esculentum* gene marker stocks for two additional generations. Each backcross generation was scored for self-incompatibility and for other segregating characteristics. Several plants of each generation then were selected for further backcrosses.

In Table 1 the segregations for self-incompatibility in the six backcross genera-

TABLE 1

*Segregation for self-incompatibility from* L. chilense *in six backcross generations to* L. esculentum *variety Tiny Tim*

| Backcross generation | Number of plants | | | Actual ratio | Chi-square for ratio of | |
|---|---|---|---|---|---|---|
| | SF | SI | Total | | 3:1 | 7:1 |
| 1 | 69 | 12 | 81 | 5.75:1 | 4.48* | 0.39 |
| 2 | 79 | 18 | 97 | 4.39:1 | 2.15 | 3.25 |
| 3 | 209 | 42 | 251 | 4.98:1 | 9.15** | 4.11 |
| 4 | 355 | 93 | 448 | 3.81:1 | 4.30* | 27.94*** |
| 5 | 61 | 21 | 82 | 2.90:1 | 0.16 | 12.88** |
| 6 | 66 | 24 | 90 | 2.75:1 | 0.13 | 19.27** |
| Total | 839 | 210 | 1049 | 4.00:1 | 13.87** | 54.22*** |

* Significant at .05 level of probability.
** Significant at .01 level of probability.
*** Significant at .001 level of probability.

tions of one *S* allele are given together with chi-square analyses. The numerical figures in some cases represent the sums of data from several smaller, similarly segregating families. The segregation for self-fertility *versus* self-incompatibility approached a 3:1 ratio although significant departures from such a ratio were found in three of the six backcross generations. The data do not show a better fit to any other simple ratio. The low heterogeneity chi-square for the 3:1 ratio ($\chi^2_h = 6.50$) indicates homogeneity of the six generations. When the actual ratios are compared, a trend toward a 3:1 ratio is apparent as self-incompatibility was progressively brought into the Tiny Tim genotype. It is evident, therefore, that in addition to an *S* allele, an independent dominant gene from *L. chilense* is necessary for the expression of self-incompatibility in Tiny Tim.

In Table 2 the data for the backcross generations to *L. esculentum* marker stocks are summarized. Data were obtained for two generations of two stocks but only one generation of the third. The most surprising feature is the difference between the two generations in segregation ratio. The abrupt shifts in ratio between backcrosses to Tiny Tim and the first backcross to *L. esculentum* and between the first and second backcross to *L. esculentum* are most easily explained on the assumption that the gene marker stocks and Tiny Tim lack certain genes

TABLE 2

*Segregation for self-incompatibility in two backcross generations to* L. esculentum *gene marker stocks*

| Marker stock | Actual BC generation | Number of plants | | | Actual ratio | Adjusted chi-square for ratio of | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SF | SI | Total | | 1:1 | 3:1 | 7:1 | 15:1 |
| *a, d, c, l* | 3 | 22 | 15 | 37 | 1.47:1 | 0.97 | 4.77 | ... | ... |
| *a, d, c, l* | 4 | 58 | 6 | 64 | 9.67:1 | ... | 39.06*** | 0.32 | 0.70 |
| *Wo, d* | 3 | 25 | 9 | 34 | 2.78:1 | 6.62 | 0.00 | ... | ... |
| *Wo, d* | 4 | 134 | 5 | 139 | 26.80:1 | ... | 36.68*** | 9.67** | 1.25 |
| *rv, al, H* | 3 | 16 | 10 | 26 | 1.60:1 | 0.96 | 1.85 | ... | ... |

necessary for the expression of self-incompatibility, but not the same genes. The segregation in the two generations was due to random assortment of these genes in the pollen since in both generations the same homozygous pistillate lines were used.

The above hypothesis assumes that four dominant genes are necessary for self-incompatibility in the recurrent parent materials. This is the least number of genes that could account for a 15 to 1 ratio. The necessary genotypes to explain these results are given in Table 3. If the hypothesis proves correct, a search for more such genes would be useful.

Inspections of the styles of the self-incompatible plants of the backcross generations revealed a segregation for strength of the reaction as shown by variable mean pollen tube lengths. Furthermore, a progressive weakening of the self-incompatibility was found to occur as backcrossing progressed. In contrast to mean pollen tube length of about 15 percent of the style in *L. chilense*, it reached about 50 percent in the fifth backcross.

The weakening of self-incompatibility in the later backcrosses to Tiny Tim was also manifest by the occurrence of plants which were intermediate with respect to self-incompatibility and self-fertility. Such plants set a few fruit containing a few seeds after self-pollination but set vigorously when cross-pollinated appropriately. These plants were classified as self-fertile, and probably account for the ratios of less than 3:1 in the fifth and sixth backcrosses (Table 1).

Four plants which were weakly self-fertile were backcrossed to Tiny Tim and the progeny were screened for self-incompatibility. Among 141 plants none were found to be strongly self-incompatible but 12 were of the intermediate type. One or more such plants were found in each of the four families. Weak self-fertility thus appears to be a valid intermediate, heritable condition but one readily confused with self-fertility.

Crosses among backcross hybrids carrying different *S* alleles usually failed due to poor pollen germination. However, pollen viability in crosses to *L. esculentum* was high. In the few successful crosses among hybrids, extreme embryo abortion was evident. As discussed later, this barrier may be due to an interaction of the *S* gene and *L. esculentum* genotype.

Since only about one fourth of the progeny of any backcross generation to

TABLE 3

*Theoretical genotypes of* L. chilense *and* L. esculentum *stocks with respect to genes necessary for self-incompatibility*

| Stock | Genotype of stock | Genotype of self-incompatible hybrid |
|---|---|---|
| *L. chilense* | $S_1S_2$ *AABBCCDD* | . . . . . |
| Tiny Tim | $S_fS_f$ *aaBBCCDD* | $S_nS_f$ *AaBBCCDD* |
| Marker stock *a, d, c, l* | $S_fS_f$ *AAbbccdd* | $S_nS_f$ *AABbCcDd* |
| Marker stock *Wo, d* | $S_fS_f$ *aaBBccdd* | $S_nS_f$ *AaBBCcDd* |
| Marker stock *rv, al, H* | $S_fS_f$ *AA------* | $S_nS_f$ *AA------* |
| All self-incompatible plants | $S_n$ *–A–B–C–D–* | $S_n$ *–A–B–C–D–* |

Tiny Tim were found to be self-incompatible, it is highly probable that about one third of the self-fertile progeny carried an *S* allele other than $S_f$. The presence of *S* alleles in a few self-fertile plants of the first backcross generation was demonstrated by successfully pollinating self-incompatible plants of the same generation but carrying the other *S* allele. However, no thorough screening for such unexpressed *S* alleles was made.

*Linkage relations:* In addition to self-incompatibility, a number of known genes and one newly discovered marker gene segregated in the backcrosses. These were dwarf ($d_1$), self-pruning (*sp*), Hairless (*H*), anthocyaninless ($a_1$), Woolly (*Wo*), lutescent ($l_1$), and potato leaf (*c*). Complete descriptions of the characteristics determined by these genes are given by RICK and BUTLER (1956). A necrotic condition segregating in the backcrosses, apparently derived from *L. chilense* was given the name Necrotic (*Nec*). The condition is first apparent as elevations or hypertrophic pustules along the veins of the leaf. In and around these growths, chlorosis and then necrosis occurs. As the leaf weakens, secondary fungus infections may occur and the leaf abscisses prematurely. The intensity of this character increased as backcrossing progressed.

All of the marker genes segregated in the expected 1:1 ratio except as noted below. In one family among five, an excess of dwarf plants was apparent. This character was difficult to score because of its modification by self-pruning and without a doubt by other minor genes. Inaccurate classification could have caused this apparent deviation. In two families from crosses to Woolly-dwarf marker stocks, Necrotic segregated in a 3:1 ratio indicating that two recessive genes controlling this character were present in the stock.

Tests of association of the marker genes, made to detect gene linkages, are summarized in Table 4. The analyses show association of self-incompatibility with dwarf, Woolly, and Necrotic. The first two of these genes are known to lie on the second chromosome of the tomato (RICK and BUTLER 1956). Tests for association of Necrotic with dwarf and Woolly place that gene also in the same

TABLE 4

*Tests for association of self incompatibility with eight marker genes*

| Gene | Recurrent parent | Backcross generations | Number of plants | | | | | Adjusted contingency chi-square |
|---|---|---|---|---|---|---|---|---|
| | | | SF + | SF marker | SI + | SI marker | Total | |
| *d* | Tiny Tim | 2, 3, 4 | 166 | 234 | 51 | 37 | 488 | 7.26** |
| *d* | Marker stocks | 4 | 164 | 159 | 21 | 0 | 344 | 17.29*** |
| *Nec* | Tiny Tim | 4, 5 | 186 | 96 | 8 | 63 | 353 | 66.33*** |
| *Nec* | Marker stocks | 4 | 208 | 57 | 4 | 11 | 280 | 18.01*** |
| *Wo* | Marker stocks | 4 | 110 | 155 | 15 | 0 | 280 | 17.77*** |
| *H* | Tiny Tim | 2, 3, 4 | 184 | 172 | 51 | 30 | 437 | 2.94 |
| *a* | Marker stocks | 4 | 34 | 24 | 3 | 3 | 64 | No analysis possible |
| *c* | Marker stocks | 4 | 23 | 35 | 2 | 4 | 64 | No analysis possible |
| *l* | Marker stocks | 4 | 29 | 29 | 1 | 5 | 64 | No analysis possible |
| *sp* | Tiny Tim | 4 | 45 | 56 | 21 | 18 | 140 | 0.63 |

linkage group. Necrotic (*Nec*) does not appear to be identical to necrosis (*ne*), a gene which in recessive condition, causes the development of a necrosis in *Cladosporium fulvum* resistant plants (LANGFORD 1948). The tentative relative positions of these five genes on the second chromosome are summarized in Figure 3.

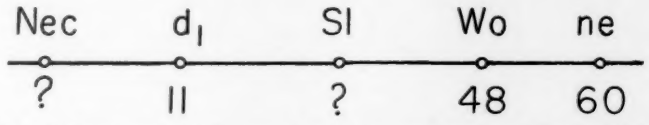


FIGURE 3.—Linkage map of chromosome 2 of the tomato showing relative positions of genes determining Necrosis and self-incompatibility.

Preliminary measurements of seven characteristics of the inflorescence and flowers of the hybrids and parent materials gave some indication that length of style, width and length of corolla segments might be associated with self-incompatibility. Length of peduncle, width and length of anther column and number of flowers per inflorescence showed no signs of such an association. Accordingly more extensive measurements were taken of the first three characteristics in a large family of the fourth backcross generation. Measurements were made of all 39 of the self-incompatible plants and of 68 of the self-fertile plants taken at random. In addition, the ratio of corolla segment width to length was calculated. The data were subjected to analyses of variance to determine the association of these characteristics with self-incompatibility and with the two linked genes, dwarf and Necrotic. The first analyses showed that Necrotic did not influence the traits in question. The data were then grouped into four phenotypic classes determined by self-incompatibility and dwarf and were reanalyzed. All analyses showed highly significant differences. Paired comparisons were then made of classes differing with respect to only one character (Table 5).

It is evident from the table that both self-incompatibility and dwarf are associated with the floral differences. Dwarf tends to shorten and narrow flower parts whereas self-incompatibility shows the opposite effects. However, the effects are by no means clear cut and interactions appear to occur. Linkage and crossing over are probably the main causes of the apparent interaction.

In the fifth and sixth backcross generations, a few self-incompatible plants occurred free of $d_1^+$, *Nec*, and the flower characteristics of *L. chilense*. These plants bore a striking resemblance to Tiny Tim. Evidently the quantitative characters studied above were associated with self-incompatibility by linkage rather than pleiotropy.

## DISCUSSION

The persistence of self-incompatibility in the backcrosses to Tiny Tim is without parallel in genetic literature. A strong resemblance of the background genotypes of *L. esculentum* and *L. chilense* is thus suggested. The chief difference with respect to incompatibility in crosses to Tiny Tim appears to reside in two

TABLE 5

*Results of the analyses of variance of four flower characteristics*

| Classes | Mean style length (mm) | Mean corolla segment width (mm) | Mean corolla segment length (mm) | Mean corolla segment ratio |
|---|---|---|---|---|
| Comparison of phenotypic classes differing in compatibility reaction | | | | |
| SI + | 9.10 | 6.32 | 9.84 | 0.644 |
| SF + | 8.86 | 5.67 | 10.53 | 0.552 |
| Difference | 0.24 | 0.65** | −0.69* | 0.092** |
| SI $d_1$ | 8.42 | 5.62 | 8.89 | 0.646 |
| SF $d_1$ | 7.06 | 4.91 | 8.07 | 0.619 |
| Difference | 1.36** | 0.71* | 0.82* | 0.027* |
| Comparison of phenotypic classes differing at the $d_1$ locus | | | | |
| SF + | 8.86 | 5.67 | 10.53 | 0.552 |
| SF $d_1$ | 7.06 | 4.91 | 8.07 | 0.619 |
| Difference | 1.80** | 0.76** | 2.46** | −0.067** |
| SI + | 9.10 | 6.32 | 9.84 | 0.644 |
| SI $d_1$ | 8.42 | 5.62 | 8.89 | 0.646 |
| Difference | 0.68* | 0.70* | 0.95* | −0.002 |

genes of major effect. The evidence that other major genes may be present in other *L. esculentum* stocks is of the utmost importance and suggests a method of tracing the phylogeny of the forms of this diverse species. Many minor genes undoubtedly affect the strength of reaction, witnessed, as backcrossing proceeded, by the increased length of pollen tubes after self-pollination and by the limited self-fertility of the $F_2$. In contrast to these results, MATHER (1943) found a rapid loss of self-incompatibility in two backcrosses of *Petunia axillaris* × *P. violacea*. He attributed his results to a well-developed polygenic difference between the species which controlled the incompatibility reaction.

The establishment of an outbreeding *L. esculentum* population at the present time appears to be difficult if not impossible, for plants of the *L. esculentum* type which carry different *S* alleles seldom cross. The barrier to the cross, manifested in poor pollen germination and embryo abortion, appears to be due to the *L. esculentum* genotype. MCGUIRE and RICK (1954) attributed the barrier found in backcrosses of $F_1$ hybrids to *L. peruvianum* to a few genes with sporophytic affect. In this case, however, the wide differences in crossability point to a multigenic control.

The association by linkage of self-incompatibility with a variety of marker genes as well as polygenes affecting the very morphological features leading to successful outcrossing (long, exserted style, and showy flowers) may not be wholly fortuitous. Such linkage associations have been shown to occur in Primula (ERNST 1936). Recently RICK (1959) has shown non random gene distribution among tomato chromosomes. An unexpectedly large number of marker genes were found on chromosome two, the chromosome on which one gene controlling self-incompatibility lies. The perpetual heterozygosity for *S* alleles in an outcrossing species such as *L. chilense* may very well lead to the development of

linked adaptive gene combinations. Both the linkage associations found here and those found by RICK (1959) could thus be easily explained.

The fact that all self-incompatible plants showed the secondary property of inhibiting the pollen of self-fertile species is consistent with the concepts of unilateral interspecific incompatibility as developed by LEWIS and CROWE (1958). Furthermore, no self-fertile plant showed this phenomenon, even among those known to carry an *S* allele. In plants with weak self-incompatibility the unilateral reaction was always present and was usually stronger than self-incompatibility. Inspection of the styles from such crosses always showed a pollen tube inhibition similar in appearance to that of self-incompatibility. Thus it appears that the two phenomena, self-incompatibility and unilateral incompatibility were properties of the same genetic and physiological system in this study.

This triple nature of the reproductive isolating barriers between the two species deserves mention here. The superficial barrier resembling self-incompatibility and preventing hybridization of *L. chilense* by *L. esculentum* was relatively easily removed by transferring *S* alleles into *L. esculentum*. A second, more potent multigenic barrier was then evident in failure of pollen germination and embryo abortion. Finally, a third, relatively simply controlled barrier, necrosis, was deleterious to hybrid survival. It is interesting that only the first of these barriers is unilateral. The more complexly controlled barrier on the other hand, was evident in reciprocal crosses. It is tempting to think that it is therefore the oldest barrier and that unilateral interspecific incompatibility is a more recent acquisition which accompanied the evolution of *L. esculentum* from a self-incompatible progenitor, via the scheme of LEWIS and CROWE (1958). It is evident that the question cannot be answered in this material for the necessary intermediate types may no longer be extant.

## SUMMARY

Evidence was collected indicating that the self-incompatibility in *L. chilense* is of the Nicotiana type.

The inheritance of self-incompatibility in hybrids of *L. esculentum* and *L. chilense* was studied in $F_1$, $F_2$, and backcross hybrids. Self-incompatibility appears to be controlled by two or more dominant genes of major action in the hybrids. A difference in *L. esculentum* stocks with respect to these genes was found. Unilateral interspecific incompatibility was shown to have the same genetic control in these materials as self-incompatibility. In addition, evidence was found of a polygenic difference between the two species affecting the strength of the incompatibility reaction. A polygenic barrier was shown to underlay the barrier due to the *S* genes. This resulted in poor pollen germination and extreme embryo abortion. A necrotic condition acting as a physiological breakdown was found among the hybrids.

Self-incompatibility was found to be associated by linkage to three genes on chromosome two and several quantitative traits. A tentative linkage map of tomato chromosome two was constructed.

Self-incompatibility was transferred from *L. chilense* to the *L. esculentum* variety Tiny Tim by six backcrosses.

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# BIOCHEMICAL DIFFERENCES BETWEEN THE MUTANTS ROSY-2 AND MAROON-LIKE OF DROSOPHILA MELANOGASTER[1]

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THE conversion of 2-amino-4-hydroxypteridine into isoxanthopterin in wild-type *Drosophila melanogaster* has been demonstrated and investigated by several groups (FORREST, GLASSMAN and MITCHELL 1956; HADORN and SCHWINCK 1956; NAWA, TAIRA and SAKAGUCHI 1958). This reaction is catalyzed by an enzyme which will also catalyze the oxidation of hypoxanthine through xanthine into uric acid, xanthopterin into leucopterin (Figure 1), and benzaldehyde into benzoic acid, all of these being typical xanthine oxidase catalyzed reactions. However, the enzyme from Drosophila requires an electron acceptor, presumed to be diphosphopyridine nucleotide (DPN), in the living organism and has therefore been called a xanthine dehydrogenase (GLASSMAN and MITCHELL 1959a).

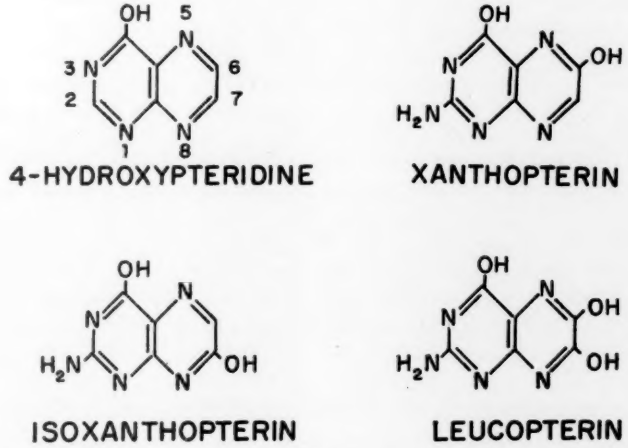


FIGURE 1.—Numbering system for the pteridine ring and chemical formulae of some of the compounds described in the text.

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[2] National Institutes of Health Predoctoral Fellow (1959–1961).

Interest concerning this enzyme arises from the fact that two mutant sites are known which affect its activity. The mutants rosy (*ry*) (HADORN and SCHWINCK 1956) and maroon-like (*ma-l*) (FORREST, GLASSMAN and MITCHELL 1956) cannot effect any of the above conversions and therefore exhibit a typical and similar phenotype (dull red eyes) and chemotype [accumulations and deficiencies of various pteridines and purines (Table 3)] (HUBBY and FORREST 1960). The only known differences between the two mutants are (1) their genetic location, (2) a "maternal effect" exhibited by young *ma-l* flies under certain breeding conditions (GLASSMAN, HUBBY and MITCHELL 1958; GLASSMAN and MITCHELL 1959b) and (3) the production of a "cross-reacting material" by the *ma-l* mutant which presumably bears some close relationship to the active enzyme (GLASSMAN and MITCHELL 1959a). The rosy mutant does not exhibit a maternal effect and produces little or no cross-reacting material. It is the purpose of this paper to present evidence for a number of additional biochemical differences between the mutants, some of which may shed light on the relationship between the mutants and on the mode of action and properties of the enzyme under their control.

In this study an allele of rosy, rosy-2 ($ry^2$), was used. The two alleles are identical phenotypically and chemotypically; however, it has recently been reported that they can be separated by crossing over (SCHALET and CHOVNICK 1960). This observation is unlikely to affect the results described in this paper in any major aspect, although obviously an investigation of both alleles in the light of the results reported herein would be of considerable interest.

This investigation arose from a parallel study of the possibility that 4-hydroxypteridine is the first pteridine product in a postulated purine → pteridine transformation. In contrast to the statements in the literature regarding the action of xanthine oxidase on simple pteridines (BERGMANN and KWIETNY 1959), it was found that in extracts of the bacterium *Azotomonas insolita* 4-hydroxypteridine was converted into 2, 4-dihydroxypteridine in a reaction catalyzed by a xanthine oxidase-type enzyme (i.e., oxygen acts as an electron acceptor) (FORREST, HANLY and LAGOWSKI 1961). The same conversion was also found to occur in wild-type Drosophila extracts, and, furthermore, unlike all of the oxidative reactions mentioned above, the reaction did not require the presence of an electron acceptor such as DPN and is therefore a xanthine oxidase-type reaction. Of even greater significance is the fact that extracts of the mutant $ry^2$ can catalyze the conversion of 4-hydroxypteridine into 2, 4-dihydroxypteridine, but extracts of *ma-l* cannot. Further investigation led to the discovery of other differences between the two mutants $ry^2$ and *ma-l* (*vide infra*), several of which are probably dependent on the activity of the "oxidase" enzyme in $ry^2$ flies and its absence in *ma-l* flies.

## METHODS AND MATERIALS

Enzyme preparations were made by grinding four-day-old adult flies (0.1 g) in a Ten Broek homogenizer with 0.02 M phosphate buffer, *p*H 7.6 (1 ml). The resultant homogenate was treated with 0.05 g activated carbon for five minutes at 4°C and then centrifuged for 20 minutes in a refrigerated Spinco, Model L,

at 20,000 rpm (Rotor No. 40). The supernatant was recentrifuged at the same speed for an additional 20 minutes. After removal of the fatty layer this final supernatant was dialyzed for three hours against 0.02 M phosphate buffer, *p*H 7.6, at 4°C. The charcoal treatment and dialysis removed endogenous pteridines, preventing their interference with assay procedures. In each preparation the protein concentration was measured using the Folin phenol reagent (LOWRY, ROSENBROUGH, FARR and RANDALL 1951) and was then adjusted to 5–7 mg/ml by dilution with 0.02 M phosphate buffer, *p*H 7.6. Reaction tubes generally contained 0.1 ml enzyme preparation, 0.1 ml substrate solution, 0.05 ml of a 1 mg/ml aqueous DPN solution (if used), and sufficient phosphate buffer (0.1 M at *p*H 7.5) to bring the final volume to 0.3 ml. Pyridoxal hydrochloride was used as a substrate at a concentration of 1 mg/ml water, while purine and pteridine substrates were made up as saturated aqueous solutions.

Reaction mixtures were allowed to incubate for two hours without agitation at 25°C and spotted directly on Whatman's No. 3 mm chromatographic paper, appropriate controls being used throughout. Chromatograms were developed in an ascending direction for 10–14 hours using a solvent composed of two parts 1-propanol:one part 6% aqueous ammonia. Compounds were located on the chromatograms by observing their fluorescence and/or absorption under ultraviolet light (principal emission 260 and/or 360 m$\mu$). Control spots of authentic compounds provided a preliminary identification of the reaction products. After elution of these products from the chromatogram using 1% aqueous ammonia, their complete identification was achieved by comparison with authentic specimens, spectrophotometrically at three different *p*H values and paper chromatographically using several different solvent systems. The difficulties encountered in the paper chromatography of some of the compounds are discussed elsewhere (FORREST, HANLY and LAGOWSKI 1961).

## RESULTS

*Pteridine oxidations:* 4-Hydroxypteridine was converted into 2, 4-dihydroxypteridine by wild-type Drosophila extracts purified by the procedure described above; however, conversion into 2, 4, 7-trihydroxypteridine did not occur until DPN was added to the preparation. In the presence of DPN, only a very small amount of 2, 4-dihydroxypteridine accumulated during the incubation period, the major product being 2, 4, 7-trihydroxypteridine. The addition of a cofactor (DPN) was also necessary for the conversions of hypoxanthine into uric acid and 2-amino-4-hydroxypteridine into isoxanthopterin by the wild-type extract. Extracts prepared from the mutant $ry^z$ did not effect xanthine dehydrogenase catalyzed reactions (in the presence of DPN) but did catalyze the oxidation of 4-hydroxypteridine into 2, 4-dihydroxypteridine without the addition of a cofactor. All of the substrates mentioned above were unchanged when added to and incubated with *ma-l* extracts.

*Oxidation of pyridoxal:* Pyridoxal (added originally with the intention of supplying a possible cofactor requirement) was converted by wild-type extracts

into a blue fluorescent compound. A quantity of this compound sufficient for identification was obtained by larger scale paper chromatography. Its ultraviolet absorption spectrum at different *p*H values was measured and compared with the corresponding spectrum of a synthetic sample of pyridoxic acid (HARRIS, HEYL and FOLKERS 1944; HUFF and PERLZWEIG 1944), revealing their close similarity (Table 1). A direct paper chromatographic comparison of the authentic and experimental materials in different solvent systems showed them to be identical (Table 2); further confirmatory evidence was obtained by paper electrophoresis and by comparison of the variation of the intensity of their fluorescences at different *p*H values (HUFF and PERLZWEIG 1944). Pyridoxamine, pyridoxine and pyridoxal phosphate were not converted into pyridoxic acid by the wild-type enzyme extract.

Extracts of the mutant $ry^2$ brought about this oxidation, but extracts from *ma-l* flies had no effect on pyridoxal.

*Miscellaneous biochemical differences:* A number of other differences between wild-type and mutant extracts were observed. In these cases, because of their complexity, the underlying reasons for the differences are obscure.

1. While investigating the possibility of using the increased absorption of DPNH over DPN at 340 m$\mu$ as a means of quantitatively studying the oxidation reactions listed above, it was found that the absorption at 340 m$\mu$ of wild-type enzyme extract itself increased with time. This extract contained 1 mg protein/ml. and, therefore, this observation does not necessarily contradict the statement made by HUBBY and FORREST (1960) that there was no increase in absorption at 340 m$\mu$ in a similar preparation, since in the latter case the protein concentra-

TABLE 1

*Ultraviolet absorption spectra in aqueous solution*

| Compound | *p*H | $\lambda_{max}$(m$\mu$) | $\lambda_{min}$(m$\mu$) |
|---|---|---|---|
| Pyridoxic acid | 1 | 315 | 264 |
| | 7 | 314 | 260 |
| | 14 | 306, 247 | 272, 237 |
| Product from action of enzyme preparation on pyridoxal | 1 | 312 | 269 |
| | 7 | 312 | 268 |
| | 14 | 306, 247 (inflection) | 273 |

TABLE 2

*Rf values in various solvents*

| Compound | 1-propanol: 6% aqueous ammonia (2:1) | 1-propanol: water (3:2) | 1-butanol: acetic acid:water (4:1:1) |
|---|---|---|---|
| Pyridoxic acid | 0.87 | 0.89 | 0.58 |
| Product from action of enzyme preparation on pyridoxal | 0.87 | 0.89 | 0.58 |

tion was 1/10 that used in these experiments. The increase in absorption was sensitive to changes in *p*H, protein concentration, and temperature and can therefore probably be attributed to an enzymatic process which may be releasing materials capable of reducing bound DPN or may be effecting release and oxidation of fully reduced, bound pteridines. The formation of such oxidation products would be expected to cause increased absorption at 340 mμ. With regard to the mutants, *ry*$^{2}$ extracts exhibited the same effect; *ma-l* extracts did not.

2. In experiments designed to investigate the occurrence of oxidase and dehydrogenase activities in larvae and pupae, it was noted that wild-type larval extracts and *ry*$^{2}$ larval extracts very rapidly darkened (2–3 minutes), becoming quite black, presumably due to melanin formation. Larval extracts of *ma-l* flies, on the other hand, remained colorless for at least 30 hours.

3. Another interesting difference between the mutants was observed while checking for the possible presence of a xanthine oxidase-type enzyme in these flies. Addition of xanthine to *ry*$^{2}$ preparations caused the formation of hypoxanthine; addition of uric acid unexpectedly caused the production of both hypoxanthine and xanthine. However, addition of uric acid to *ma-l* preparations led only to the production of hypoxanthine. On the other hand, the addition of uric acid to wild-type preparations caused the formation of neither compound. Using $C^{14}$-labeled uric acid (uric acid-6-$C^{14}$, Isotope Specialties Co., Inc.), it was established that the hypoxanthine and xanthine produced in these reactions came from endogenous sources and not from the added uric acid. Since the conversion of hypoxanthine into uric acid is blocked in both of these mutants, different pathways for the production of both xanthine and hypoxanthine must be assumed. The reason why uric acid affects these pathways, and does so only in the mutants, remains obscure.

Table 3 summarizes the differences outlined above and those reported by other investigators among wild type, *ry*$^{2}$ and *ma-l D. melanogaster*.

## DISCUSSION

The reactions described in this paper probably do not have physiological significance, except possibly the conversion of 4-hydroxypteridine into 2, 4-dihydroxypteridine. This conversion may have some significance for pteridine biosynthesis, although this still remains to be proved. The point to be emphasized, however, is that the *ry*$^{2}$ mutant is capable of carrying on oxidative reactions and the *ma-l* mutant is not. It is possible, of course, that this oxidative activity has no connection with the effect of the rosy gene on the enzyme xanthine dehydrogenase. However, in view of the similarity of the substrates and the nature of the oxidation, it seems reasonable to presume that oxidation is due to residual activity in the enzyme affected by this gene.

Two theories may be advanced to explain the differences observed between the mutants. (1) The wild-type allele of *ma-l* could control formation or incorporation of a cofactor necessary for complete activity of the enzyme xanthine dehydrogenase. In the absence of this cofactor the enzyme would be completely

TABLE 3

*The differences and similarities between* ma-l *and* ry$^2$

| | Wild type | *ry*$^2$ | *ma-l* |
|---|---|---|---|
| 2, 4-Di- → 2, 4, 7-tri-hydroxypteridine (DPN) | + | − | − |
| 2-Amino-4-hydroxypteridine → isoxanthopterin (DPN) | + | − | − |
| Xanthopterin → 2-amino-4, 6, 7-trihydroxypteridine (DPN) | + | − | − |
| Hypoxanthine → xanthine (DPN) | + | − | − |
| Xanthine → uric acid (DPN) | + | − | − |
| 4-Hydroxy- → 2, 4-dihydroxy-pteridine | + | + | − |
| Pyridoxal → pyridoxic acid | + | + | − |
| "Fast melanin production" | + | + | − |
| "340 mμ optical density increase" | + | + | − |
| Maternal effect | − | − | + |
| Endogenous hypoxanthine* | − | + | + |
| Endogenous hypoxanthine and xanthine* | − | + | − |
| Lack of isoxanthopterin | − | + | + |
| Reduced amounts of red pigments | − | + | + |
| Biopterin accumulated | − | + | + |
| 2-Amino-4-hydroxypteridine accumulated | − | + | + |
| Sepia pteridines accumulated | − | + | + |
| CRX Activity | +++ | ± | ++ |

* Hypoxanthine and/or xanthine produced from endogenous sources when uric acid is added to enzyme preparation.

inactive. The *ry*$^2$ allele, on the other hand, might be concerned with the synthesis of the apoenzyme, and, in the mutant condition, it would produce a modified enzyme still active in an oxidative capacity. A disadvantage of this theory is that it has been shown that *ma-l* extracts contain a protein capable of reacting with specific antibodies for xanthine dehydrogenase [cross-reacting (CRX) material]; *ry*$^2$ extracts contain little or no material of this nature. (GLASSMAN and MITCHELL 1959a). It seems rather unlikely that the alteration of an enzyme molecule (e.g., by the mutant *ry*$^2$ allele) which still leaves the enzyme oxidatively "active" would abolish its cross-reacting activity. On the basis of the presence or absence of cross-reacting material in the mutants, and the maternal effect exhibited by the *ma-l*$^+$ allele, GLASSMAN and MITCHELL (1959b) postulated the gene sequence shown in Figure 2a as the most likely arrangement. This scheme would scarcely be tenable on the above "cofactor" theory.

It should also be pointed out that added cofactors [flavinadenine dinucleotide, flavin mononucleotide, molybdenum or iron, some of which are known cofactors for milk xanthine oxidase (*cf.* DE RENZO 1956)] caused no observable effects in extracts of either mutant, nor have mixing experiments between mutant extracts produced any enzyme activity (GLASSMAN and MITCHELL 1959a). Although none of these experiments can be considered to be conclusive—for example, the cofactors might be tightly bound and not exchangeable with exogenous material —they provide cogent arguments against the "cofactor" theory.

(2) Xanthine dehydrogenase could have two sites of enzyme activity, each

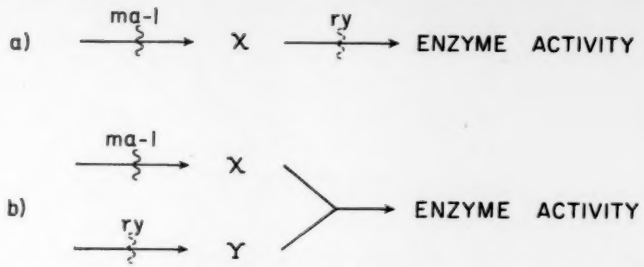


FIGURE 2.—Schemes proposed by GLASSMAN and MITCHELL (1959b) for the action of the two genes in the production of xanthine dehydrogenase.

controlled by a different gene. [Dual functions for the classical xanthine oxidase and chicken liver xanthine dehydrogenase, where purines, pteridines and aldehydes are oxidized at one site and reduced DPN is oxidized at another site, have been suggested by other data (*cf.* DE RENZO 1956)]. Thus, the mutant $ry^2$ would affect the dehydrogenase site (perhaps the site where the cofactor is bound), leaving the site of oxidation unaffected but probably with a markedly reduced efficiency. On the other hand, the mutant *ma-l* would necessarily affect the site of substrate binding, consequently interrupting both oxidase and dehydrogenase activities. Alteration of the substrate site would be assumed to have little or no effect on the protein's ability to react with specific antibodies.

This explanation would be more in accord with the second of GLASSMAN and MITCHELL's schemes for the action of the two genes on xanthine dehydrogenase activity (Figure 2b). Thus, *ma-l* flies would contain the product of the wild-type *ry* allele, *y*, having all of the cross-reacting ability but no enzyme activity. In contrast, $ry^2$ flies would contain the product of the wild-type *ma-l* allele, *x*, having no cross-reacting ability, but which would be enzymatically active with an efficiency, however, less than that of wild-type flies. In fact, by an extension of this scheme, it becomes somewhat analogous to the system in *Escherichia coli* controlling tryptophan synthetase (CRAWFORD and YANOFSKY 1958), where two proteins, A and B, are necessary to make a fully functional enzyme, although each, by itself, has some enzyme activity in the half reactions involved in the production of tryptophan. Cross-reacting activity, in contrast to this, is confined to one of the proteins (LERNER and YANOFSKY 1957). The analogy with the scheme proposed in this paper for the mutants $ry^2$ and *ma-l* is at once apparent.

To test these hypotheses purification of the proteins involved in the different enzyme activities is contemplated.

## SUMMARY

In addition to the known differences between the mutants *ma-l* and *ry*, both of which lack a functional xanthine dehydrogenase, a number of new biochemical differences between the two have been discovered. The most significant of these

is the ability of extracts of the $ry^2$ mutant, like those of wild type, to catalyze the conversion of 4-hydroxypteridine into 2, 4-dihydroxypteridine and of pyridoxal into pyridoxic acid without the addition of DPN. Extracts from *ma-l* flies are unable to catalyze these reactions. Two theories have been advanced to explain these observations: (1) the "cofactor" theory in which it is assumed that the *ma-l* mutant is lacking an essential cofactor and the $ry^2$ mutant has an altered apoenzyme; (2) the "dual site" theory in which it is assumed that each mutant allele affects a different site on the enzyme molecule, the *ma-l* mutant having a lesion at the site of substrate binding and the $ry^2$ mutant at the site of dehydrogenase activity. An extension of this latter theory, to bring it into line with previous work on these mutants, suggests that these sites may be on different protein molecules, both of which are necessary for complete enzyme activity; one of the protein molecules contains cross-reacting activity, and the other contains residual oxidase activity.

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ADDENDUM

A referee has criticized the work reported herein on the basis that the mutants studied are not from coisogenic stocks. Admitting the validity of the criticism, we would like to point out the very great difficulties involved experimentally in attempting to carry out enzyme work on true coisogenic stocks of *Drosophila melanogaster*. We have now approached the problem from the opposite viewpoint in the following way. Two genetically different alleles of rosy, and one of maroon-like present in quite different genetic backgrounds have been shown to behave in exactly the same way as rosy-2 and maroon-like respectively. Thus, from random sources, the mutants all show their characteristic biochemical properties as described in this paper. It seems reasonable to assume, therefore, that their biochemical activities are, indeed, connected with their known genetic defects.

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# A STABILIZER OF ANTIGENIC PHASES IN SALMONELLA ABORTUS-EQUI[1]

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IN diphasic strains of Salmonella, the flagellar antigen type oscillates between phase-1 and phase-2. The phenomenon has been known as phase variation. Its genetic mechanism was studied in detail by LEDERBERG and IINO (1956). The frequency of phase variation is generally as high as $10^{-5}$ to $10^{-3}$ per cell per division (STOCKER 1949); but a much lower frequency of the alternation has been observed in certain Salmonella serotypes, e.g. in *S. abortus-equi* and *S. paratyphi A* (BRUNER and EDWARDS 1941; EDWARDS and BRUNER 1939). In the present paper, a genetic factor is reported which controls the low frequency of phase variation in *S. abortus-equi*.

## MATERIALS AND METHODS

As a stable phase strain, *S. abortus-equi* CDC-26 was used. The strain is stable in phase-2, *enx* type, which is determined by $H_2^{enx}$. The presence of the suppressed phase-1 antigen type determinant $H_1^a$ in a phase-2 clone was demonstrated by transduction of $H_1^a$ as well as $H_2^{enx}$, to various recipients (LEDERBERG and EDWARDS 1953) and by rare spontaneous change to the stable phase-1 type (LEDERBERG, personal communication). The cells of CDC-26 move rather slowly in both phases being even slower in phase-1 than in phase-2. Strain TM2 of *S. typhimurium* was used for comparison as a diphasic type. TM2 expresses *i* type in phase-1 and *1.2* type in phase-2 (designated by *i*: *1.2*). The change from phase-2 to phase-1 in TM2 occurs at the rate of $3 \times 10^{-4}$ per cell per division in a broth culture, and at about one fourth of that from phase-1 to phase-2.

The phase-1 antigen type of a stable phase-2 strain was determined by linked transduction of $Fla_1^+$ and $H_1$ from that strain to *S. paratyphi B* SW666. SW666 is a strain without flagella originated from phase-1 (*b* type) monophasic *S. paratyphi B*, by mutation of $Fla_1^+$ to $Fla_1^-$, which is linked to $H_1$. The hidden phase-1 antigen type of the stable phase-2 strain is detected in some recombinants of the transduction.

The general procedures of cultivation, transduction and selection of serotypic recombinants were according to the methods of LEDERBERG and IINO (1956).

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Phage PLT22 was used as the vector in transduction (ZINDER and LEDERBERG 1952). The selection of alternative phases was carried out by the semisolid nutrient gelatin agar (NGA) tube method described by EDWARDS and EWING (1955). Antigen types were determined by slide agglutination.

## RESULTS AND CONCLUSIONS

*Phase stability of* S. abortus-equi: A stock culture of CDC-26 was streaked on a nutrient agar plate. After 24 hour incubation at 37°, each of 20 isolated colonies was transferred to broth and cultivated overnight on a rotator; the culture reached about a concentration of $2 \times 10^8$ cells per ml. Two tenths ml of each broth culture was then dropped in a NGA tube supplemented with anti-*enx* serum. The amount of anti-*enx* serum was so adjusted as to prevent the moving of *enx*-type cells but not that of the cells of other antigen types (EDWARDS and EWING 1955). A series of phase-2 cultures of TM2 was prepared in the same way and 0.2 ml samples were likewise dropped into an NGA tube supplemented with anti-*1.2* serum. In parallel, another 0.2 ml was dropped from each of both CDC-26 and TM2 cultures into a plain NGA tube. They were incubated at 37° C and observed day by day. In all of the tubes, CDC-26/plain, TM2/plain and TM2/anti-*1.2* serum, grown cells moved into NGA and formed swarms within two days after they were inoculated. The antigen types of the cells of those swarms were *enx* in CDC-26/plain, *1.2* in TM2/plain and *i* in TM2/anti-*1.2* serum tubes. In CDC-26/anti-*enx* tubes, however, no swarms were detected even after five days prolonged cultivation. The experiments were repeated five times, and the same results were obtained each time: no swarms were produced in any CDC-26/anti-*enx* tubes but they were produced from all others.

To test the possibility that nonmotile type cells are produced in phase-1, broth cultures of CDC-26 were spread on NGA plates and the segregation of two colonial types, compact and swarm, was examined by the same method as the test of $Ah_1^-$ (IINO 1961). Regular segregation as in $Ah_1^-$ strains was not observed in CDC-26.

Thus, the phases of *S. abortus-equi* CDC-26 are exceedingly stable in contrast to those of a diphasic strain *S. typhimurium* TM2. The stable type phase and the unstable type are easily identified by NGA tube cultures.

Spontaneous changes of phase have occurred from *enx* to *a* in *S. abortus-equi*, but only very rarely (EDWARDS and BRUNER 1939). One *a* clone was obtained by anti-*enx* NGA selection from the *enx* types of *S. abortus-equi*, with the strain SL23 in the course of an experiment conducted for other purpose. The *a* type was as stable as the original *enx* type, and only one *enx* type swarm was detected from 160 anti-*a* NGA tube cultures.

*Transductional analysis of the phase-stability determinant:* Flagellar antigen types of Salmonella are determined by the $H_1$ and $H_2$ genes. One or the other of these genes is expressed in a given clone which is described as being in phase-1 and phase-2 respectively. A factor inseparable from $H_2$ is the phase determinant, alternating between two states: $H_2$ active vs. $H_2$-inactive-$H_1$ active. In order

to test whether the difference in the stability of $H_2$ is caused by its own intrinsic structure or by other genetic factor(s), transductions were carried out from an *enx* type culture of CDC-26 to an *i* type culture of TM2. Serotypic recombinants were isolated from NGA plates supplemented with anti-*i* and anti-*1.2* sera.

Among the recombinants isolated, four were diphasic *a:1.2* type, 42 diphasic *i:enx* and 19 monophasic *enx* (Table 1). That *i* is the hidden phase-1 of the last

TABLE 1

*Transductions between* S. typhimurium *TM2 and* S. abortus-equi *CDC-26. Hidden antigenic phases of diphasic strains or monophasic strains were parenthesized or bracketed respectively*

| Donor | Recipient | Selection on | Recombinant Type | Recombinant No. | Transduced loci |
|---|---|---|---|---|---|
| CDC-26 | TM2 | anti-i, 1.2 | a : 1.2 | 4 | $H_1^a$ |
| [a]: enx | i : (1.2) | NGA | i : enx | 42 | $H_2^{enx}$ |
| | | | [i]: enx | 19 | $H_2^{enx}\,Vh_2^-$ |
| | | | Total | 65 | |
| TM2 | CDC-26 | anti-enx | [a]: 1.2 | 38 | $H_2^{1.2}$ |
| (i): 1.2 | [a]: enx | NGA | a : 1.2 | 15 | $H_2^{1.2}\,Vh_2$ |
| | | | Total | 53 | |

type was demonstrated on three PLT22 sensitive clones by transduction of the $H_1^i$ to *S. paratyphi* B. SW666. Those results show that *a* and *enx* are each transduced from the phase-2 culture of CDC-26. When *a* is transduced the resulting transduction remains diphasic, whereas when *enx* is transduced some transductional clones become monophasic. By anti-*enx* serum selection, *i* phase cultures were rarely obtained from the *enx*-monophasic transductional clones. The *i* phase cultures obtained were also monophasic. The stabilization of the inherent $H_2$ activity in *S. abortus-equi* CDC-26 is therefore caused by a factor which is linked to $H_2$.

The *a:1.2*- and *i:enx*-diphasic and *enx*-monophasic transductional types obtained are almost as motile as the recipient strain TM2, which moves into NGA media three to four times faster than CDC-26. The slow motility of CDC-26, therefore, depends principally on factors independent of $H_1$, $H_2$ and the phase stabilizer.

The reciprocal transductions, from an *i* type culture of TM2 to an *enx* type culture of CDC-26, support the above conclusion (Table 1). Among 53 transductional clones screened on anti-*enx* NGA plates, 38 were stable *a* and 15 were diphasic *a:1.2*. The hidden antigens of the *a* clones are presumed to be *1.2*. The frequencies of linked transduction of the $H_2$ stability controller with $H_2$ agree well between the reciprocal transductions (about 30 percent). The $H_2$- stability controller will be given a symbol $Vh_2$. Its allele in TM2 is $Vh_2^+$ and $Vh_2^-$ in CDC-26.

## DISCUSSION

The present study demonstrated that the genetic factor $Vh_2$, which is closely linked to $H_2$ but separable from it, regulates the frequency of antigenic phase

variation in Salmonella. In other words, $Vh_2$ controls the change of the inherent state of $H_2$ activity. A gene which regulates mutability of other genes has been reported in some bacterial strains (TREFFERS, SPINELLI and BELSER 1954; SKAAR 1956; MIYAKE 1960). They have been called mutator genes. In contrast to those mutator genes, the function of $Vh_2$ as far as we know is the specific control of $H_2$ activity. However, no comprehensive test has been made of mutation rates in $Vh_2^+$ and $Vh_2^-$ strains. Analogues to the $H_2$–$Vh_2$ systems are perhaps found in the variegation of higher organisms (LEWIS 1950), for instance the *Ac–Ds* and *Mp* elements in corn (MCCLINTOCK 1956; BRINK and NILAN 1952) and the suppression of phenotypic effect by transposition of euchromatic genes to heterochromatic regions in Drosophila (DEMEREC 1940; BAKER 1953).

A similar type of monophasic behavior as that of *S. abortus-equi* has been reported in *S. paratyphi A* (BRUNER and EDWARDS 1941; EDWARDS, BARNES and BABCOCK 1950). *S. paratyphi A* isolated from nature is usually in *a* phase (phase-1). The *a* phase is very stable and an alternative phase, phase-2 (*1.5* phase), can be obtained only through selection by anti-*a* serum. The phase-2 culture, either isolated from nature or obtained from a phase-1 culture, is also stable and rarely reverts to phase-1. The monophasic property of *S. paratyphi A* may be caused by stabilization of $H_2$ as in *S. abortus-equi*.

A strain that carries $Vh_2^-$ is highly stable in either phase, and a phase stable strain of a desired antigen type may be obtained by transduction. Consequently, $Vh_2^-$ strains are excellent materials for the production of specific flagellar antigens (EDWARDS and EWING 1955).

## SUMMARY

The frequency of antigenic phase variation in Salmonella is regulated by the genetic factor $Vh_2$ which is closely linked to $H_2$; that is, $Vh_2$ controls the stability of $H_2$ state. In a strain of *Salmonella abortus-equi*, CDC-26, an allele $Vh_2^-$ stabilizes $H_2$ in its existing state, whether inactive or active, and produces phase-1 or phase-2 monophasic types respectively.

## ACKNOWLEDGMENT

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# ANOMALOUS HOMOLOGY OF FLAGELLAR PHASES IN SALMONELLA[1]

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PHASE-1 and phase-2 antigen types of a diphasic salmonella strain are determined by distinct genes, $H_1$ and $H_2$, respectively (LEDERBERG and EDWARDS 1953; LEDERBERG and IINO 1956). They are nonallelic and are transduced independently. Therefore, an H-antigen type recombinant obtained by a transduction usually expresses the antigen type of the donor in one phase, but retains that of the recipient in the other. The present paper deals with a genetic analysis of an abnormal recombinant type which expresses phase-1 types of both donor and recipient.

## MATERIALS AND METHODS

*Salmonella typhimurium* SW1061, the original strain of the phase-1 duplicate type reported in the present paper, is an $H_1$-inactive mutant derived from TM2 (STOCKER, ZINDER and LEDERBERG 1953). This wild-type strain expresses *i* antigen in phase-1 and *1.2* in phase-2 (designated by *i:1.2*). SW1061 does not produce flagella in phase-1, though it carries the *i*-antigen type determinant (designated by *0(i):1.2*). The genotype of SW1061 is symbolized by $Fla_1^+$-$Ah_1^-H_1^iH_2^{1.2}$ (IINO 1961a). Genetic markers of the remaining salmonella strains, *S. abony* CDC-103 *b:enx* and *S. heidelberg* SL28 *0(r:1.2)*, used in the present experiment are $Fla_1^+Ah_1^+H_1^bH_2^{enx}$ and $Fla_1^-Ah_1^+H_1^rH_2^{1.2}$, respectively (LEDERBERG and IINO 1956). $Fla_1$, $Ah_1$ and $H_1$ are closely linked with each other and are transduced simultaneously in high frequency.

The general procedures of cultivation, transduction and selection of serotypic recombinants were conducted according to the methods of LEDERBERG and IINO (1956). Phage PLT22 was used exclusively as the mediator in transductions.

Anti-H sera were prepared by the method of EDWARDS and EWING (1955). Antigen type was determined qualitatively by slide agglutination test with antisera of titer 100, and quantitatively by tube agglutination test.

## RESULTS AND CONCLUSIONS

In a transduction experiment from CDC-103 to SW1061 (IINO 1961a), a transductional clone which alternates phases between *b* and *0* (flagellaless) was

[1] Contribution from the National Institute of Genetics, Japan. No. 389. This work was supported by a research grant from the National Institute of Allergy and Infectious Diseases (E-2872), Public Health Service, U.S.A.

obtained (Table 1). The mixture of a broth culture of the *0:b* clone with the lysate of a phase-2 culture of TM2 was brushed on an NGA plate supplemented with anti-*b* serum as a selective agent. Twenty swarms which developed as a result of H-transductions were isolated and typed for their antigen and alternative phase. Seven clones among them were agglutinated by anti-*i* serum, and after anti-*i* serum selection produced the alternative phase which is agglutinated by anti-*b* serum. These *i:b* type clones do not dissociate into motile and nonmotile type any more. The remaining 13 clones were all agglutinated by anti-*1.2* serum. An alternative H phase has not been obtained from these clones by anti-*1.2* serum selection, but the dissociation of *0* type was observed in NGA plates. These results are formulated as follows:

CDC-103 *b:enx* —x SW1061 *0(i):1.2* → *0(i):b*
TM2 *i:1.2* —x *0(i):b* → *i:b* & *0(i):1.2.*

Thus the abnormal type of recombinant obtained in the first transduction has lost the phase-2 antigen of the recipient and expresses the phase-1 antigen type of both donor and recipient alternatively. The phase-1 antigen acquired from the donor was not replaced by phase-1 antigen but phase-2 antigen in the second step transduction.

The identity of *b* antigens of the *i:b* type and the donor strain, CDC-103, was examined by cross agglutination and absorption experiments. Antisera were prepared against 0.5 percent formalin-saline suspensions of CDC-103 and the *i:b* clone. Anti-CDC-103 serum and anti-*i:b* serum were absorbed by *i:enx* cells obtained from *S. typhimurium* TM2 *i:1.2* —x CDC-103 *b:enx*, and anti-*b* (CDC-103) and anti-*b* (*i:b*) sera were obtained, respectively. The agglutination titers of anti-*b* (CDC-103) serum against cultures of both CDC-103 and *i:b* types were identical (24,000). After complete absorption of anti-*b* (CDC-103) serum by the *i:b* cultures, the serum did not agglutinate the cells of CDC-103 any more. In parallel, anti-*b* (*i:b*) serum showed the same titer (26,000) against both CDC-103 and the *i:b* type cells, and it was completely absorbed by the cells of CDC-103. Thus, as far as the agglutination reaction is concerned, the *i:b* type has identical *b* antigen as CDC-103.

In order to test the linkage of *i* and *b* determinants of the *i:b* type, a phage PLT22 lysate of an *i:b* clone culture was mixed with a broth culture of SL28 and brushed on NGA plates. The swarms grown as a result of $Fla_1^+$ transduc-

TABLE 1

*Transduction from a diphasic strain of* S. abony, *CDC-103* b:enx *to SW1061* 0(i):1.2. *Transductional clones were screened on NGA plates supplemented with anti-*1.2 *serum*

| Antigen type phase-1 : phase-2 | Number of transductional clones in Expt. 1 | Expt. 2 | Number of reversional clones: control for expt. 1 |
|---|---|---|---|
| b:1.2 | 95 | 23 | 0 |
| 0:enx | 84 | 13 | 0 |
| i:1.2 | 4 | 1 | 1 |
| 0:b | 0 | 1 | 0 |

tion were isolated and their antigen types were examined. Twenty-nine among 39 of those transductional clones were *r:1.2*. The remaining ten clones were *i:1.2* type, which was expected from the simultaneous transduction of $Fla_1$ and $H_1$. These two were the sole recombinant types, and no antigen type which contains *b* was obtained (LEDERBERG and IINO 1956, ¶ 3.5). From these results it is concluded that the antigen-*b* determinant of the *i:b* clone is not transduced linked to $Fla_1$ and $H_1{}^i$.

Transduction was next carried out from a *b*-phase culture of the *i:b* type clone to an *a*-phase (phase-1) culture of a diphasic strain SW925 *a:enx*. Antigen type recombinants were screened on NGA plates supplemented with anti-*a* and anti-*enx* sera. Among 44 recombinant clones, 37 expressed *i* and *enx* in phase-1 and phase-2, respectively. The remaining seven clones expressed *a* and *b*-antigen types alternatively. No recombinant showing *1.2* type was detected. The frequency of the alternative expression of *a* or *b*-antigen types is the same as that of phase variation in SW925. Thus, the *b* determinant was transduced and replaced with $H_2$ of the recipient.

In conclusion, the *b* determinant of the *i:b* type clone behaves exclusively as an allelic locus to $H_2$. The possibility that a hidden $H_2{}^{1,2}$ is present in the phase-1 antigen duplicate type is excluded by the antiserum selection and the transduction tests.

## DISCUSSION

The possibilities of an $H_1$-duplication or a heterogenote of $H_1$ in the phase-1 duplicate strain reported in this paper are excluded for the following reasons: (1) allelism between $H_2$ and the *b*-type determinant and (2) the absence of $H_2{}^{1,2}$ in the strain. The phenomena are best explained by the assumption that the duplication of phase-1 antigen occurred by an unequal recombination resulting in the replacement of $H_2{}^{1,2}$ by $H_1{}^b$. The new type now behaves as if it were $H_1{}^i\ H_2{}^b$.

The occurrence of such an unequal recombination indicates a structural homology between $H_1$ and $H_2$. $H_1$ and $H_2$ might have a certain degree of synaptic affinity. The chance of synapsis of $H_1$ and $H_2$ in transduction of these genes may be very rare, as the phase-1 duplicate type reported in the present paper is the only occasion of unequal recombination out of over 3,000 serotypic recombinants which have been examined in our laboratory for the various transductional analyses of salmonella H antigens. The behavior of the translocated *b*-antigen determinant as an allele of $H_2$ indicates that the barrier of synapsis between $H_1$ and $H_2$ are not their own structural differences, but may be the differences in other genes involved in a transductional fragment together with $H_1$ or $H_2$. The genes which have been known to be transduced simultaneously with $H_1$ are $Fla_1$ (STOCKER *et al.* 1953; LEDERBERG and IINO 1956) and a methylator of lysine in flagellin molecules (STOCKER, MCDONOUGH and AMBLER 1961). The genes homologous to these $H_1$-linked genes are not present in the $H_2$ region, instead a stability controller of antigenic phases, $Vh_2$, is closely linked to $H_2$ (IINO 1961b).

The genetic homology of $H_1$ and $H_2$ leads us to a speculation on their phylo-

genic relations: one of them might have originated by duplication and translocation of the other; the structural differentiation might have occurred between then thereafter. These postulates trace the process of evolution from the primary monophasic serotype to the diphasic type. The secondary monophasic types might have originated from the latter by inactivation of either $H_1$ or $H_2$ (IINO 1961a). The occurrence of $H_1$ or $H_2$ duplication in nature is indicated from the isolation of $H_1$-duplicate strains (LEDERBERG 1961) or of so called triphasic strains (reviewed by KAUFFMANN 1954).

The frequency of the alternative expression of two antigen types in the phase-1 duplicate strain is the same as that in the original transductional recipient strain SW1061: the *b* determinant translocated to the $H_2$ locus becomes unstable and changes its activity at the frequency specific to the $H_2$ locus of the strain. The stability of the $H_2$ state is regulated by $Vh_2$ which is closely linked to $H_2$ (IINO 1961b). Consequently the present results suggest that the presence of an antigen determinant in the vicinity of $Vh_2$ is essential for its action on $H_2$.

## SUMMARY

An abnormal H-antigen type recombinant which alternatively expresses phase-1 antigens of both donor and recipient was obtained from a transduction between *Salmonella typhimurium* SW1061 and *S. abony* CDC-103.

The duplicate phase-1 antigen type determinant $H_1^b$ of the recombinant behaves as an allelic locus of phase-2 antigen type determinant $H_2$. The recombinant is presumed to be originated by an unequal recombination: $H_2$ locus is replaced by $H_1$ of the donor in the transduction. The phylogenic relation between $H_1$ and $H_2$ was discussed.

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# A DUPLICATION OF THE $H_1$ (FLAGELLAR ANTIGEN) LOCUS IN SALMONELLA[1]

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TYPICAL Salmonella strains are "diphasic" with respect to their flagellar antigens. That is, they manifest one of two genes, $H_1$ or $H_2$, but not both in a given clone until a phase variation takes place and the alternative antigen is expressed.

The genetic analysis of this alternation by transductional methods (LEDERBERG and EDWARDS 1953; LEDERBERG and IINO 1956) has shown that the variation depends on a "change of state" at the $H_2$ locus. The alternating factor has not been separated by recombination from the determinant for antigenic specificity of $H_2$. On the other hand, repeated cycles of alternation in state have occurred at $H_2$ without influencing its antigenic specificity. The $H_1$ gene, the determinant of the antigen of the alternative phase, is not linked to $H_2$, but the expression of $H_1$ depends on the inactive state of $H_2$. The present paper is part of a comprehensive study of the genetics of Salmonella phase variation (IINO 1958, 1961a,b).

Various anomalies, or exceptions to the simple pattern of diphasic variation have occurred, whose assimilation to the general scheme can throw light on the genic control of protein synthesis. The anomaly presented in this paper is represented by CDC-157, a strain of *Salmonella paratyphi B*, now much used as an antigen for the production of diagnostic anti-*1.2* antiserum (EDWARDS and BRUNER 1942). The advantage of CDC-157 for this purpose is its apparent monophasic behavior; that is, large clones will consist nearly exclusively of cells whose flagellar antigen is *1.2*. A genetic analysis of this strain has revealed two anomalies: (1) The *1.2* antigen is determined by an allele at the $H_1$ locus instead of the $H_2$ usual for this antigen, and (2) the stock is carrying a duplicate $H_1$ locus. The genotypic formula of CDC-157 is thus $H_1{}^bH_1{}^{1.2}$, by contrast to the genotype $H_1{}^bH_2{}^{1.2}$ for typical strains of *S. paratyphi B*. These genotypes accurately summarize the genetic behavior of the respective strains.

## MATERIALS, METHODS AND RESULTS

The basic techniques of transductional analysis and background information on Salmonella immunogenetics have been detailed in a previous paper (LEDER-

[1] These studies were mainly conducted at the Department of Genetics, University of Wisconsin in 1953. DR. TETSUO IINO's association with them has done much to clarify the issues brought up here. The work was supported by research grants from the National Cancer Institute, U.S. Public Health Service (C-2157) and the National Science Foundation.

BERG and IINO 1956). To recapitulate, a phage grown on a strain A may carry individual markers, or a small cluster of closely linked markers, to strain B, an operation symbolized A—x B or its equivalent B x—A. The transduced markers replace their homologues in the recipient, an event usually detected by selection against the recipient marker. In this case specific antiserums are used to immobilize cells of the recipient flagellar serotype. The $H_1$ and $H_2$ loci are transduced independently, as if unlinked to one another, but $H_1$ is linked to certain *Fla*⁻ (flagellaless, nonmotile) factors. The frequency of transduction is so low, $10^{-5}$ per marker per phage, that unlinked markers are never, in practice, found together in the same transduction clone.

Strain CDC-157 was kindly furnished by DR. P. R. EDWARDS, of the Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia, and he has also searched his archives for information on its history. A group of some 25 strains was isolated about 1942 from an outbreak of gastroenteritis in the Canal Zone, and identified as *S. paratyphi B*, var. *java*, i.e., monophasic *b* and d-tartrate-positive, phage type 3b. Attempts to select alternative phases by selection with *b*-antiserum met with sporadic success (EDWARDS and BRUNER 1946). CDC-157 itself was such a selection from a culture designated as N25. Further attempts by EDWARDS and BRUNER, and by us to repeat such a selection from this culture, have been unsuccessful, and the two cultures may be accurately designated as monophasic *1.2* and *b* respectively. Another culture, N97, had been isolated from the same outbreak, and also designated as *b*. After it was received from DR. EDWARDS in 1953, however, this culture and single-colony reisolations sporadically generated *1.2* phases after selection in *b* antiserums. The analysis reported here has therefore concentrated on N97 (see Table 1). The sluggishness of phase variation of N97 and its derivatives has undoubtedly been the chief obstacle to this work.

Other strains and techniques have been fully described in previous publications

TABLE 1

*History of strains N25, N97 and CDC-157*

| Original strains from Canal Zone 1942 epidemic | b antiserum selection gave | Then 1:2 antiserum selection gave | Then b antiserum selection gave |
|---|---|---|---|
| N25 b tested <1946 | 1.2 phase CDC-157 | 0 | . . |
| N25 b tested 1953 | 0 | . . | . . |
| N97 b tested 1953 | 8/8 colonies 1.2 phases | 8 tests: 0<br>2 tests: b phases | 0 |

(STOCKER, ZINDER and LEDERBERG 1953; LEDERBERG and IINO 1956; LEDERBERG 1956). Most of the cultures stem from the type collection in DR. EDWARDS' laboratory (EDWARDS and BRUNER 1942). Many of the specific sera were kindly furnished from the same source and by the Standards Laboratory, Central Public Health Laboratory, Colindale, London. DR. EDWARDS has also confirmed the serotypes of the principal cultures described here.

*Variation in strain N97:* Upon selection in anti-*b* agar each of eight single colonies of N97 (*b*) gave *1.2* phases, though many of the tubes showed no swarms for several days. But only two of a total of ten isolated *1.2* phases, which may be abbreviated N97 (*b*; *1.2*), would revert to *b* (*b*; *1.2*; *b*). The phases were refractory to further attempts at demonstrating phase variation. Similar trials with N25(*b*) gave no *1.2* phases, though CDC-157 had been isolated some years before as a stable *1.2* variant from N25 (*b*). The following cultures are therefore available for further study: the original N25 (*b*) and N97 (*b*), presumably of common origin; the N25 (*1.2*) secured in 1946 and N97 (*1.2*) recently obtained from N97 (*b*). At the present time, the N25 cultures are stubbornly monophasic, while variation from *b* $\rightleftarrows$ *1.2* can be demonstrated with some difficulty in N97 cultures.

*Transductions with N25 (1.2) = CDC-157:* The present study of this strain stems from the findings of transduction experiments that its *1.2* antigen is consistently inherited and at the $H_1$ rather than at the $H_2$ locus (Table 2). For example, in transductions from CDC-157 to *S. abony* $H_1{}^{b}H_2{}^{enx}$ (symbolized CDC-157—x *abony*) the progeny was *1.2:enx*, a serotype which does not occur naturally. This suggests that the *1.2* antigen of CDC-157 is homologous with the *b* ($H_1{}^{b}$) rather than the *enx* ($H_2{}^{enx}$) antigen of *S. abony*. By contrast, transductions of the *1.2* antigen to *S. abony* from typical diphasic strains such as *S. typhimurium* or *S. paratyphi B* have led to the serotype *b:1.2*, supporting the homology of the typical $H_2{}^{1.2}$ with $H_2{}^{enx}$. These results are different but unambiguous depending entirely on the specific strain used as donor. The other transductions of Table 3 furnish additional support for the formulation of CDC-157

TABLE 2

*Transductions from strain CDC-157, presumptive genotype* $H_1{}^{1.2}H_2{}^{-}$

| CDC-157 —x: | Genotype $H_1$ | Genotype $H_2$ | Progeny serotype | | Inferred genotype $H_1$ | Inferred genotype $H_2$ |
|---|---|---|---|---|---|---|
| a *S. paratyphi* B. SW666 | *b* | .. | 1.2: | .. | *1.2* | .. |
| b *S. typhi* H901 | *d* | .. | 1.2: | .. | *1.2* | .. |
| c *S. abony* | *b* | *enx* | 1.2: | enx | *1.2* | *enx* |
| d N25 (b) | *b* | .. | 1.2: | .. | *1.2* | .. |
| e *S. miami* | *a* | *1.5* | 1.2: | 1.5 | *1.2* | *1.5* |
| Compare typical *S. paratyphi B*, strain CDC-3, as donor, presumptive genotype $H_1{}^{b}H_2{}^{1.2}$ | | | | | | |
| CDC-3—x: | | | | | | |
| cc *S. abony* | *b* | *enx* | b: | 1.2 | *b* | *1.2* |
| ee *S. miami* | *a* | *1.5* | b: | 1.5 | *b* | *1.5* |
| | | | a: | 1.2 | *a* | *1.2* |

TABLE 3

*Transductions to CDC-157* (1.2: –) *as recipient*

| CDC-157 x—: | Genotype $H_1$ | Genotype $H_2$ | Progeny serotype | | Inferred genotype $H_1$ | Inferred genotype $H_2$ |
|---|---|---|---|---|---|---|
| a. *S. abony* | | | *b*: | . . | *b* | . . |
| b. | *b* | *enx* | 1.2: | enx | *1.2* | *enx** |
| c. *S. typhimurium* | *i* | *1.2* | *i*: | . . | *i* | . . |
| d. *S. paratyphi* B. SW666 | *b* | . . | *b*: | . . | *b* | . . |

* Further tests were made on this derivative:
3b $H_1^{1.2}H_2^{enx}$ x— *S. typhimurium* $H_1^{i}H_2^{1.2} \rightarrow H_1^{i}H_2^{enx}$ viz., $H_1^{1.2}$ was again replaced by $H_1^{i}$.

as $H_1^{1.2}$. The results of the preceding sections might be explained on the assumption that $H_1^{b}$ had undergone mutation to $H_1^{1.2}$ rather than phase variation. The recurrence of the change N97 $b \rightarrow$ N97 (*1.2*) cast some doubt on the mutation hypothesis and provoked further study of these strains.

*Transduction with N97 (b) and N97 (1.2) as donors (see Table 4):* These experiments verify the results already shown with the corresponding strains of N25 and support the assignment of $H_1^{b}$ and $H_1^{1.2}$ to the two phases. N97 therefore displays both $H_1^{b}$ and an unusual $H_1^{1.2}$ in various clones. Two hypotheses might be considered, (1) an anomalous duplication of $H_1$ in the genotype $H_1^{b}H_1^{1.2}$ or (2) a remarkable rate and direction of mutation at the $H_1$ locus in a euhaploid: $H_1^{b} \rightleftarrows H_1^{1.2}$. These alternatives could be distinguished by experiments with N97 as recipient.

*Transductions to N97 as recipient:* These results (Table 5) disqualify the mutation hypothesis and support the duplication of $H_1$. By a series of single substitutions, N97 (*b*) engenders the following homologous serotypes: *b:1.2* $\rightarrow$ *b:i* $\rightarrow$ *b:a* $\rightarrow$ *c:a*. The last form now carries two standard $H_1$ alleles from type strain. The mutation hypothesis could not account for the transition of $H_1^{b}$ to $H_1^{1.2}$, $H_1^{i}$, and $H_1^{a}$ respectively in the first three cases. We may conclude that N97 (*b*) and N97 (*1.2*) are respectively $\boldsymbol{H_1^{b}}H_1^{1.2}$ and $H_1^{b}\boldsymbol{H_1^{1.2}}$.

Table 6 also displays further tests of these genotypes in transductional confrontations. The production of *b:c* as well as *a:c* from $H_1^{c}$—x $H_1^{a}H_1^{b}$ indicates the equivalence of the two $H_1$ loci in the recipient.

*An* $H_2$ *locus in N97:* No $H_2$ phase could be elicited from N97 by selection in *b* or *1.2*-antiserum, nor did transductions from N97—x *S. abony* ($H_2^{enx}$) or *S.*

TABLE 4

*Transductions with N97* (b) *and N97* (1.2) *as donor*

| N97 (b) —x: | Genotype $H_1$ | Genotype $H_2$ | Progeny serotype | | Inferred genotype $H_1$ | Inferred genotype $H_2$ |
|---|---|---|---|---|---|---|
| a *S. typhimurium* | *i* | *1.2* | b: | 1.2 | *b* | *1.2* |
| b *S. lomalinda* | *a* | *enx* | b: | enx | *b* | *enx* |
| c *S. miami* | *a* | *1.5* | b: | 1.5 | *b* | *1.5* |
| N97 (1.2)—x | | | | | | |
| d *S. abony* | *b* | *enx* | 1.2: | enx | *1.2* | *enx* |

*typhimurium* ($H_2^{1.2}$) give any evidence of an $H_2$ factor in the donor. However, $H_2$ factors can be transduced in an active form only when the donor strain itself is expressing the $H_2$ factor (LEDERBERG and IINO 1956). Therefore, it cannot be excluded that N97 (*b*; *1.2*) has the constitution, say, $H_1^bH_1^{1.2}H_2^{1.2}$, the expression of $H_2$ being subject to an usually sluggish alternation of phase by analogy with $H_1^aH_2^{enx}$ in *S. abortus-equi* (IINO 1961a).

Explicit evidence for an $H_2$ locus in N97 comes from the introduction of $H_2^{enx}$ by transduction into N97 and its derivatives (Tables 5, 6). Such isolates showed frequent alternation of phase between $H_2^{enx}$ and one (only one) of the manifest

TABLE 5

*Transductions with N97* (b) *as recipient*

| | N97 (b) x— | Genotype $H_1$ | Genotype $H_2$ | Progeny serotype | | Inferred genotype $H_1$ | Inferred genotype $H_1$ | Inferred genotype $H_2$ |
|---|---|---|---|---|---|---|---|---|
| a | | | | i: | b | *i* | *b* | .. |
| | *S. typhimurium* | *i* | *1.2* | | | | | |
| b | | | | i: | 1.2 | *i* | *1.2* | .. |
| c | | | | b: | enx | *b* | (*1.2*) | *enx* |
| | *S. abony* | *b* | *enx* | | | | | |
| d | | | | 1.2: | enx | *1.2* | (*b*) | *enx* |

TABLE 6

*Transductions with N97 derivatives*

| | Derivative | —x or x— | Salmonella: | Progeny serotype | | Inferred genotype $H_1$ | Inferred genotype $H_1$ | Inferred genotype $H_2$ |
|---|---|---|---|---|---|---|---|---|
| a | 5a* $H_1^iH_1^b$ | x— | *sendai* $H_1^aH_2^{1.5}$ | a: | b | *a* | *b* | .. |
| b | | | | b: | c | *b* | *c* | .. |
| | 6a $H_1^aH_1^b$ | x— | *altendorf* $H_1^cH_2^{1.7}$ | | | | | |
| c | | | | a: | c | *a* | *c* | .. |
| d | | | | a: | enx | *a* | (*c*) | *enx* |
| | 6c $H_1^aH_1^c$ | x— | *abony* $H_1^bH_2^{enx}$ | | | | | |
| e | | | | c: | enx | (*a*) | *c* | *enx* |
| f | | | | 1.2: | enx | *1.2* | (*i*) | *enx* |
| | 5b $H_1^iH_1^{1.2}$ | x— | abony $H_1^bH_2^{enx}$ | | | | | |
| g | | | | b: | 1.2 | *1.2* | *b* | .. |
| h | 5a $H_1^bH_1^i$ | —x | *miami* $H_1^aH_2^{1.5}$ | i: | 1.5 | *i* | .. | *1.5* |
| i | | | | a: | 1.2 | *a* | .. | *1.2* |
| | 6a $H_1^aH_1^b$ | —x | *typhimurium* $H_1^iH_2^{1.2}$ | | | | | |
| j | | | | b: | 1.2 | *b* | .. | *1.2* |

* Symbols 5a, 6a, etc. refer to the progeny listed in Tables 5 and 6.

$H_1$ factors. If we assign the genotype $H_1^a H_1^c H_2^{enz}$, the phase variability of $H_2^{enz}$ would be expected to accord with that of its diphasic provenience. However, the control of phase between two $H_1$ loci, $H_1^a$ and $H_1^c$ is not now understood and is difficult to study when it occurs so rarely in the parental cultures as well as the progeny.

The alternative suggestion must be considered that $H_2^{enz}$ has substituted for an $H_1^a$ to give the diphasic *c:enx*, $H_1^c H_2^{enz}$ (by analogy with the case reported by IINO 1961b). This cannot be disputed except for the strict separation of recognized $H_1$ and $H_2$ factors in all other tests reported here. The *c:enx* type was tested —x $H_1^i H_2^{1.2}$ and in the face of some technical problems gave one successful transduction typed as *i:enx* as expected for a regular $H_2^{enz}$ factor.

*Are the duplicated factors linked?* In a number of technically favorable trials, including several listed here, the $H_1$ factors of N97 derivatives were inherited independently. Further tests were made —x SW666 $Fla_1^-$ (Table 7) that indicated linkage of each $H_1$ to $Fla^+$. However, the $Fla^+$ might also have been duplicated and we conclude nothing as to the mutual linkage of the duplicated $H_1$'s. Indeed, there is nothing that would firmly contradict a model of complete duplication of the genome, i.e., that N97 is diploid, though $H_1$ is the only locus known to be heterozygous.

TABLE 7

*Linkage of* $H_1$ *factors to* $Fla_1$

*Transductions of N97 derivatives (Table 6) to SW-666*

| SW-666 $Fla_1^-$ $H_1^b$ x— | Serotypes of $Fla^+$ selections | Inferred genotypes $Fla_1$ | $H_1$ | $H_1$ |
|---|---|---|---|---|
| 6c $H_1^a H_1^c Fla^+$ | b: . . | + | *b* | . . |
| in phase a | a: . . | + | a | (*c*?) |
| 6c $H_1^a H_1^c Fla^+$ | b: . . | + | *b* | . . |
| in phase c | c: . | + | *a* | (*c*?) |

Pooled $Fla^+$ swarms, selected for motility without antiserum, were streaked out, and also selected in b antiserum. The isolated clones were tested and typed as shown.

## DISCUSSION

The chief interest of these findings is that an unlikely anomaly still fits the predictions of the genetic model of phase variation with strict separation of $H_1$ and $H_2$ homologues. An exception to this rule has been found only by IINO (1961b) who has traced an allele that behaves as $H_2^b$ from an $H_1^b$ parent. It seems most plausible that $H_2$ originally arose as a duplication of $H_1$, and still retains some fundamental homology with it, though this cannot usually be demonstrated. Indeed, synaptic homology should depend on the regional quality of a chromosome segment, beyond a single gene (ZINDER 1960).

The phenotypic confusion of $H_1^{1.2}$ and $H_2^{1.2}$ is paralleled by the *lw* antigen. *Salmonella wien* must be typed as $H_1^b H_2^{lw}$, and *S. dar-es-salaam* as $H_1^{lw} H_2^{enz18}$ to interpret the findings of EDWARDS, DAVIS and CHERRY (1955). In contrast to *1.2*, *lw* has long been familiar to Salmonella immunologists as behaving in some

strains as a phase-1, in others as phase-2. The inference is suggested by the pattern of alternative phases and is given genetic meaning by the transduction tests.

SPICER and DATTA (1959) have reported a clone of *S. typhimurium* as giving unstable transductional derivatives which show evidence of diploidy at both the $H_1$ and $H_2$ loci. It differs from the present case in part in the irreproducibility of the effect and transduction of the duplicated $H_1$ locus or loci to other strains.

In typical diphasic strains, we can envisage that the $H_1$ gene is repressed by the action of the $H_2$ gene, or perhaps of a variable phase controller immediately adjacent to it. No evidence of a controller at the $H_1$ locus has been found in typical diphasic $H_1H_2$ strains, only in the response of $H_1$ to $H_2$ control. This system has evidently broken down in the $H_1H_1$ duplications; but we cannot say whether a residual $H_2$ controller is still coupled to one $H_1$ or whether another gene is pinch hitting for the evident regulation.

Apart from the troublesome obstinacy of N97 in undergoing phase variation, genetic analysis by selective transduction has evident disadvantages for the articulation of a complex system. We may look forward to constructive applications of sexual breeding methods in Salmonella immunogenetics (BARON, CAREY and SPILMAN 1959).

## SUMMARY

*Salmonella paratyphi B* strain CDC-157 has the genotype $H_1{}^bH_1{}^{1,2}H_2{}^r$; typical strains of this serotype are $H_1{}^bH_2{}^{1,2}$. Two anomalies are thus evident, the duplication of $H_1$ and the representation of the *1.2* antigen at the $H_1$ locus. The transductional progeny of CDC-157 affirm the assigned genotype. They include such exceptional serotypes as *1.2:1.5*, *1.2:enx*, *b:i*, *b:c*, and *a:c*.

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# RECOMBINATION BETWEEN SUPPOSEDLY HOMOLOGOUS CHROMOSOMES OF GOSSYPIUM BARBADENSE L. AND G. HIRSUTUM L.[1]

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HISTORICALLY, comparative cytological studies of related species are based on observations of meiotic pairing in interspecific hybrids. Counts are made on the numbers of paired bivalents at Metaphase I, polyvalent associations when present are recorded, and if the material is technically suitable these observations are supplemented by chiasmata counts in diplotene and metaphase associations. In genera such as Gossypium, in which many interspecific combinations can be studied, the cytological evidence so obtained provides a valuable and independent check on evolutionary relationships determined by classical taxonomic analysis.

Yet the phenomenon of preferential pairing in the polyploid derivatives of interspecific hybrids, first reported by NEWTON and PELLEW (1929), shows that the amount of bivalent pairing in an interspecific hybrid which contains only one genome from each parent is a crude measure, at best, of relative cytological affinity. In the colchicine derived tetraploid hybrid in which each parental genome occurs twice, pairing is competitive, and the relative amounts of homologous and heterologous association provide more refined measurements of intergenomic affinity. Some years ago, it occurred to the writer that relative pairing affinity could be expressed quantitatively by the appropriate use of genetic markers (STEPHENS 1950). GERSTEL and PHILLIPS successfully expanded this idea to develop a systematic study of several marker loci and applied it to the analysis of polyploids derived by colchicine treatment from many of the interspecific hybrids available in Gossypium (see GERSTEL and PHILLIPS 1958; also PHILLIPS 1961b for general summaries of their extensive survey). They were able to show that several of the American diploid species which exhibit similar meiotic pairing behavior (13 II + 13 I) in triploid hybrids with tetraploid *G. hirsutum*, give characteristically different amounts of preferential pairing in the corresponding hexaploid derivatives. Presumably these differences are related to degree of structural divergence between the chromosomes of similar but not homologous genomes. Slight, but significant amounts of preferential pairing have been found in the colchicine derived tetraploid hybrids of the Asiatic diploid species, *G. arboreum* L. and *G. herbaceum* L. (PHILLIPS 1961a). Evidence of

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preferential pairing in polyploid hybrid derivatives of *G. hirsutum* L. and *G. barbadense* L. is still lacking.

It had been suspected for some time that linked genes recombine less freely in segregating progenies of interspecific hybrids than they do in intraspecific segregations. This situation was noted by SILOW (1944) in the case of *arboreum* × *herbaceum* hybrids and was recently confirmed by PHILLIPS (1961a) in a carefully controlled test. Further, RHYNE (1958) showed that the insertion of a chromosomal segment from *arboreum* into a genetically marked and supposedly homologous region in *hirsutum* produced a change in linkage map distance. He pointed out that the effect might be due to general suppression of recombination in the affected chromosome or alternatively to a shift in chiasma position. These findings indicate that conditions which satisfy the requirements of normal pairing do not necessarily satisfy the requirements of normal recombination. It is perhaps justifiable to assume that the "recombination test" provides a more sensitive index of chromosomal homology than the "preferential pairing test," though its application is restricted to chromosomes marked with two or more loci.

The experiments to be described are concerned with a study of recombination between supposedly homologous chromosomes in *barbadense* and *hirsutum*. The segment within which recombination was studied is over 90 crossover units in length and includes five linked marker loci. Since in both species, chiasma frequencies at metaphase average around two per bivalent, it is likely that the marked segment includes most of the genetic map length of the chromosome. (For further discussion of this point, see STEPHENS 1955). The multiply marked segment allows recombination to be studied simultaneously in adjacent chromosomal regions, thus enabling the investigator to distinguish between general crossover suppression and shifts in crossover frequencies.

## MATERIALS AND METHODS

The marked segment of the *hirsutum* chromosome which was studied in these experiments included the following loci:

| | |
|---|---|
| $N$ — naked seed | $n$ — fuzzy seed |
| $Lc_1$ — brown lint | $lc_1$ — white lint |
| $Yg_2$ — normal green leaf | $yg_2$ — yellow-green leaf |
| $R_2$ — petal spot | $r_2$ — spotless |

Their serial order and relative crossover distances had been determined previously (STEPHENS 1955). The linkage map is drawn to scale in Figure 1a.

Because of the normal bivalent pairing and fertility in *hirsutum* × *barbadense* hybrids it is usually assumed that all these loci are common to both species. Surprisingly enough, considering the attention which has been given to the comparative genetics of these species, the critical tests of allelism and/or location in a linkage group are only available for the $R_2$ locus (HARLAND 1929) and for the $Yg_2$ locus (RHYNE 1955). Homology in the other loci, $N$ and $Lc_1$, is still only a reasonable assumption. Thus naked seeded mutants in *barbadense* are recessive,

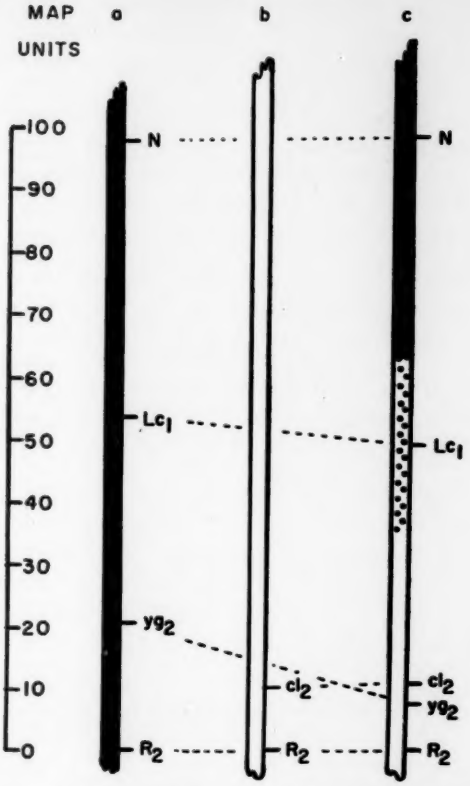


FIGURE 1.—Relative maps of supposedly homologous chromosomes of *barbadense* and *hirsutum* and their recombinant. (a) *hirsutum*, (b) *barbadense*, (c) recombinant obtained in the third backcross, *barbadense* to *hirsutum*. *Hirsutum* segments shown in black, *barbadense* in white, unknown origin, spotted.

not dominant as in *hirsutum*, and their comparative genetics are still obscure. No brown lint mutant at the $Lc_1$ locus has yet been found in *barbadense*.

Again, the recessive mutant, short-branch ($cl_2$) occurs in *barbadense*, and is known to be linked with $R_2$ with ten percent crossing over (SILOW 1946). No short-branch mutant has been found in *hirsutum* but it is usually assumed that this species carries a homologous locus with a stable normal allele, "$Cl_2$". On this assumption, the "$Cl_2$" locus in *hirsutum* should be placed either ten units below $R_2$ in the *hirsutum* map shown in Figure 1a, or else above $R_2$ and closely linked with $Yg_2$. From evidence to be presented later, it will be shown that the latter placement is correct. The linkage map of the supposedly homologous *barbadense* chromosome, on the basis of identical gene order, is shown in Figure 1b.

The crossing procedure which was adopted to analyze recombination within the marked chromosome is summarized in Figure 2. The primary *hirsutum* parent was an Upland marker stock, SM8, which carries the linked combination, *N—Lc*$_1$*—"Cl*$_2$*"—yg*$_2$*—r*$_2$. Phenotypically it has naked seeds, brown lint, normal fruiting habit, yellow-green leaves and spotless petals. The *barbadense* parent was an Egyptian strain, kindly provided by DR. S. O. S. DARK, Shambat Experiment Station, Sudan. Phenotypically, it has tufted (i.e. nonnaked) seeds, white lint, short-branch fruiting habit, normal green leaves and large petal spot. It should, therefore, carry the homologous linked combination, *"n"—"lc*$_1$*"—cl*$_2$*—Yg*$_2$*—R*$_2$. The $F_1$ was backcrossed to a multiple recessive *hirsutum* tester stock, SM4, having the linked combination, *n—lc*$_1$*—"Cl*$_2$*"—yg*$_2$*—r*$_2$. Although, theoretically, the recessive short-branch character should not have appeared in the first backcross generation, about one third of the plants exhibited the short-branch phenotype with varying degrees of penetrance. This suggested that *cl*$_2$, when transferred to a *hirsutum* background might show reversed dominance relationships in a manner previously described by HARLAND (1934) in the case of his "Crinkled Dwarf" transferences. It was planned, therefore, to select from the backcross the linked combination, *N—Lc*$_1$*—cl*$_2$*—Yg*$_2$*—R*$_2$, and to test recombination in a second backcross to SM4. The second backcross was made and the progeny raised before it was discovered that a white-linted (*lc*$_1$) selection had been made in error. This left the *Lc*$_1$ locus unmarked in the second backcross, but in any case the expression of the short-branch character was too mild and too variable for accurate scoring to be performed. Further selections were made in this

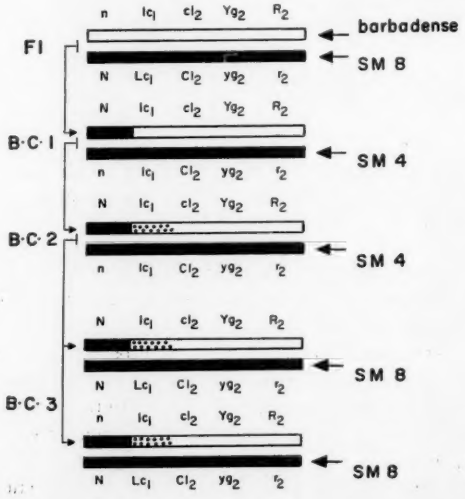


FIGURE 2.—Diagram of crossing program used to study recombination between supposedly homologous chromosomes of *barbadense* and *hirsutum*.

generation, therefore, for plants with well-expressed short-branch characteristics, normal green leaves and large petal spot. Some of the selections had naked, others fuzzy seeds. All were backcrossed to SM8, representing a third backcross to *hirsutum*.

Seven third-backcross families were grown from which five plants were selected for further study. Short-branch expression was still variable, both between and within families, indicating that dominance reversal was incomplete. Furthermore, there was a deficiency of short-branch segregates in all families. The five plants selected were all heterozygous for the *barbadense* segment, $cl_2$—$Yg_2$—$R_2$, and heterozygous for $Lc_1$. Three of the plants were heterozygous and two homozygous for $N$. The selfed families from these five selected plants provided the material for studies of recombination. Reference to the last two linkage diagrams in Figure 2, which represent the cytogenetic constitutions of the third backcross selections, shows that recombination in the interval, $cl_2$—$R_2$ would involve crossing over between *barbadense* and *hirsutum* segments (heterospecific recombination). Recombination in the interval between $N$ and some position to the left of $Lc_1$ would involve exchanges between *hirsutum* segments only (homospecific recombination). Between these intervals there is a "no man's land" on either side of, and including, the $Lc_1$ locus. In this region recombination would involve exchanges between a *hirsutum* segment and a segment of unknown, and possibly mixed origin. This dubious region was a consequence of the mistake made in the selection of the parent for the second backcross, which has been noted earlier.

Because difficulty was anticipated in the scoring of short-branch, the selfed seeds from each of the five selections were divided into approximately equal lots, and duplicate families raised from them in successive seasons. This duplication under different seasonal conditions provided a check on scoring accuracy. As will be shown later, scoring proved to be reasonably consistent. The instability of *barbadense* petal spot, $R_2$, on transference to *hirsutum* created a more difficult problem and some loss of information. In the selfed progenies of the third backcross, somatic mutation was very common. It was expressed as an erratic reduction in size or complete disappearance of the petal spot in many $R_2$ segregates. An individual plant might produce varying proportions of spotted and spotless flowers; frequently the petals of a single flower might differ in presence or absence or size of spot. There was a general tendency for the proportion of spotless flowers to increase as the plants grew older, but early scoring did not solve the problem, because some plants which had been repeatedly scored as spotless would eventually produce a flower with a faint spot. It was necessary, therefore, to attempt daily scorings of each individual plant which had previously been scored as spotless, until a flower with a spot was observed. Only those plants in which a spotted petal had never been recorded were classified as spotless ($r_2$). In spite of the intensive scoring system used, the unstable families produced a large excess of apparently spotless segregates. It is probable that many $R_2$ segregates had mutated to $r_2$ before flowering commenced. Attempts to stabilize spot expression by selecting apparently stable $R_2$ plants were unsuccessful because somatic mutation re-

curred in their progenies. In the absence of counterselection, few spotted forms would have survived past the third backcross generation.

## RESULTS

The five third-backcross selections were all heterozygous for the marked segment, $Lc_1$—"$Cl_2$"—$Yg_2$, and their selfed progenies provided information on recombination in this region. Only three of the five selections were appropriately marked in the $N$—$Lc_1$ segment, while in the $yg_2$—$r_2$ segment analysis was complicated by the instability of petal spot. For these reasons, it will be convenient to present the data on recombination from each of the three regions separately.

*Recombination in the* $Lc_1$—"$Cl_2$"—$yg_2$ *region:* The complete data for this region obtained in the seasons, 1959 and 1960, are presented in Table 1. Tests of homogeneity between seasons and between families were made. As shown at the right of the table, in none of the five families was there significant hetero-

TABLE 1

*Segregations obtained in the selfed progenies of five selections from third backcross families* (barbadense *to* hirsutum) *involving recombination in the* $Lc_1$–"$Cl_2$"—$yg_2$ *region. Dominant alleles (including* $cl_2$ *which shows reversed dominance in* hirsutum) *are indicated by* (+), *recessive alleles by* (−).

| Family | $Lc_1$ / $cl_2$ / $Yg_2$ | | | | | * | * | | | Total | $\chi^2(7)$ | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | + | − | + | − | + | − | + | − | | | |
| | | + | − | + | − | − | + | − | + | | | |
| | | + | − | − | + | − | + | + | − | | | |
| S5580–39 | | | | | | | | | | | | |
| 1959 | | 71 | 5 | 2 | 1 | 29 | 32 | 4 | 1 | 145 | | |
| 1960 | | 57 | 5 | 1 | 2 | 17 | 35 | 3 | 0 | 120 | | |
| Total | | 128 | 10 | 3 | 3 | 46 | 67 | 7 | 1 | 265 | 4.4 | 0.70–0.80 |
| S5582–7 | | | | | | | | | | | | |
| 1959 | | 96 | 4 | 3 | 0 | 31 | 31 | 4 | 0 | 169 | | |
| 1960 | | 61 | 4 | 0 | 1 | 23 | 22 | 1 | 0 | 112 | | |
| Total | | 157 | 8 | 3 | 1 | 54 | 53 | 5 | 0 | 281 | 4.8 | 0.50–0.70 |
| S5582–16 | | | | | | | | | | | | |
| 1959 | | 87 | 7 | 3 | 1 | 28 | 40 | 2 | 3 | 171 | | |
| 1960 | | 61 | 8 | 1 | 0 | 13 | 34 | 0 | 1 | 118 | | |
| Total | | 148 | 15 | 4 | 1 | 41 | 74 | 2 | 4 | 289 | 6.0 | 0.50–0.70 |
| S5582–36 | | | | | | | | | | | | |
| 1959 | | 71 | 4 | 3 | 0 | 30 | 50 | 3 | 1 | 162 | | |
| 1960 | | 67 | 0 | 1 | 1 | 11 | 26 | 3 | 0 | 109 | | |
| Total | | 138 | 4 | 4 | 1 | 41 | 76 | 6 | 1 | 271 | 13.8 | 0.05–0.10 |
| S5582–38 | | | | | | | | | | | | |
| 1959 | | 93 | 5 | 1 | 0 | 21 | 35 | 5 | 0 | 160 | | |
| 1960 | | 38 | 4 | 1 | 0 | 15 | 21 | 3 | 0 | 82 | | |
| Total | | 131 | 9 | 2 | 0 | 36 | 56 | 8 | 0 | 242 | 3.3 | 0.80–0.90 |
| GRAND TOTAL | | 702 | 46 | 16 | 6 | 218 | 326 | 28 | 6 | 1348 | | |

Heterogeneity between families: $\chi^2(28) = 34.0$, $P = 0.20$–$0.30$.

* Parental classes starred.

geneity between the 1959 and 1960 scorings, though in the case of Family S5582-36 the chi-squared value is only slightly above the five percent level. Nor were there significant differences between families (see foot of table), so that it was permissible to calculate linkages from the grand totals.

The individual gene segregations and their paired linkage relationships are analyzed in Table 2. The former, shown at the top of the table deviate widely

TABLE 2

*Analyses of individual gene segregations and linkage relationships in the* $Lc_1$—"$Cl_2$"—$yg_2$ *region*
*Data from Table 1*

| | | | Total | $\chi^2(1)$ | P |
|---|---|---|---|---|---|
| Individual gene segregations | | | | | |
| Expected (3 : 1) | | 1011:337 | 1348 | ... | ..... |
| $Lc_1$:$lc_1$ | | 964:384 | 1348 | 8.7 | 0.001–0.01 |
| $cl_2$:"$Cl_2$" | | 1050:298 | 1348 | 6.0 | 0.01 –0.02 |
| $Yg_2$:$yg_2$ | | 1062:286 | 1348 | 10.3 | 0.001–0.01 |
| Linkages | | | | | |
| $Lc_1\ cl_2$ | $Lc_1$ "$Cl_2$" | $lc_1\ cl_2$ | $lc_1$ "$Cl_2$" | Total | C.O. percent |
| 718 | 246 | 332 | 52 | 1348 | $38.8 \pm 2.3$ |
| $cl_2\ Yg_2$ | $cl_2\ yg_2$ | "$Cl_2$" $Yg_2$ | "$Cl_2$" $yg_2$ | | |
| 1026 | 22 | 34 | 264 | 1348 | $4.5 \pm 0.6$ |
| $Lc_1\ Yg_2$ | $Lc_1\ yg_2$ | $lc_1\ Yg_2$ | $lc_1\ yg_2$ | | |
| 730 | 234 | 332 | 52 | 1348 | $39.6 \pm 2.3$ |

* Parental classes starred.

from the expected Mendelian ratios. That these deviations are attributable to differential transmission or survival rather than inaccurate scoring is indicated by the fact that the deficient class is in each case associated with the allele introduced from the *hirsutum* parent. That is, the segment, $Lc_1$—"$Cl_2$"—$yg_2$, as a whole is deficient. The deficiency is satisfactorily explained by the weak growth and late flowering of all $yg_2$ types.

Crossover percentages were calculated both by the method of maximum likelihood and by the recombination method (see MATHER 1938). As the two methods yielded estimates which differed in all cases by less than two percent, the maximum likelihood estimate has been adopted throughout this study. The crossover values given in the lower part of Table 2 show that "$Cl_2$" and $yg_2$ are closely linked and that the probable map sequence is $Lc_1$—"$Cl_2$"—$yg_2$.

*Recombination in the* N—$Lc_1$ *region:* The complete data obtained from three families in the 1959 and 1960 seasons are presented in Table 3. Segregations obtained in the 1959 plantings were homogeneous, but in 1960 the duplicate plantings of the same families showed heterogeneity. Further analysis (see foot of Table 3) showed that the lack of agreement was chiefly due to the linkage component of heterogeneity. The individual gene segregations and linkage relationships are analyzed in Table 4. It is obvious that the 1960 plantings of Family

TABLE 3

*Segregations obtained in the selfed progenies of three selections from third backcross families* (barbadense *to* hirsutum) *involving recombination in the* N—$Lc_1$ *region*

| Family | $N^*Lc_1$ | $N\ lc_1$ | $n\ Lc_1$ | $n^*lc_1$ | Total |
|---|---|---|---|---|---|
| 1959 | | | | | |
| S5580–39 | 75 | 24 | 31 | 15 | 145 |
| S5582–16 | 92 | 40 | 28 | 11 | 171 |
| S5582–36 | 73 | 37 | 34 | 18 | 162 |
| Total | 240 | 101 | 93 | 44 | 478 |
| | | $\chi^2(6) = 7.0$; P = 0.30–0.50 | | | |
| 1960 | | | | | |
| S5580–39 | 66 | 33 | 12 | 9 | 120 |
| S5582–16 | 50 | 33 | 25 | 10 | 118 |
| S5582–36 | 70 | 17 | 12 | 10 | 109 |
| Total | 186 | 83 | 49 | 29 | 347 |
| | | $\chi^2(6) = 16.2$; P = 0.01–0.02 | | | |

Components of heterogeneity in 1960 season

| Source | d.f. | $\chi^2$ | P |
|---|---|---|---|
| *N* vs. *n* | 2 | 5.6 | 0.05–0.10 |
| $Lc_1$ vs. $lc_1$ | 2 | 4.1 | 0.10–0.20 |
| Linkage | 2 | 6.5 | 0.02–0.05 |
| Total | 6 | 16.2 | 0.01–0.02 |

* Parental classes starred.

S5582-36 differ markedly from the others in linkage relationships. Excluding this planting, the crossover estimates do not differ significantly from independence—their combined estimate is 49.1 percent. The "aberrant planting" yields a significantly different estimate of 33.9 percent. No satisfactory explanation can be offered for this discrepancy, which involves an apparent difference in linkage relationships between duplicate plantings of the same family.

*Recombination in the* "$Cl_2$"—$yg_2$—$r_2$ *region:* In the third backcross, the expression of the short-branch character was too variable for accurate scoring, but four families were scored satisfactorily for leaf color and petal spot. It was in this generation that spot mutability first occasioned difficulty in scoring, and many of the plants scored as $R_2$ had become phenotypically $r_2$ by the end of the flowering period. However, as shown in Table 5, the individual gene segregations were in accordance with Mendelian expectation, the families were homogeneous, and a combined estimate of 8.2 percent crossovers was obtained.

Selfed families from five selected plants in the third backcross generation were grown in duplicate plantings in 1959 and 1960. The extent to which the somatic instability of petal spot would interfere with scoring was not appreciated in 1959 and no reliable estimates were obtained. In 1960, a system of daily scoring throughout the flowering period was initiated as explained earlier in this paper. In one of the five families, no petal spot appeared although its parent had been scored as $R_2$. In a second family, only ten percent of the plants produced flowers

with a faint and barely recognizable spot, and it was, therefore, useless for obtaining a linkage estimate. The remaining three families segregated as shown in Tables 6, 7 and 7a.

TABLE 4

*Analyses of individual gene segregations and linkage in the* N–$Lc_1$ *region. Data from Table 3*

| | Individual gene segregations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Family | $N$:$n$ | | Total | $\chi^2(1)$ | P | $Lc_1$:$lc_1$ | | Total | $\chi^2(1)$ | P |
| 1959 | | | | | | | | | | |
| Pooled data from three families | 341 | 137 | 478 | 3.4 | 0.05–0.10 | 333 | 145 | 478 | 7.3 | 0.001–0.01 |
| 1960 | | | | | | | | | | |
| S5580–39 | 99 | 21 | 120 | 3.6 | 0.05–0.10 | 78 | 42 | 120 | 6.4 | 0.01 –0.02 |
| S5582–16 | 83 | 35 | 118 | 1.4 | 0.20–0.30 | 75 | 43 | 118 | 8.2 | 0.001–0.01 |
| S5582–36 | 87 | 22 | 109 | 1.4 | 0.20–0.30 | 82 | 27 | 109 | 0.0 | 0.90 –0.95 |

| | Linkage | | | | | |
|---|---|---|---|---|---|---|
| Family | $N^*Lc_1$ | $N\,lc_1$ | $n\,Lc_1$ | $n^*lc_1$ | Total | C.O. percent |
| 1959 | | | | | | |
| Pooled data from three families | 240 | 101 | 93 | 44 | 478 | 47.8 ± 3.4 |
| 1960 | | | | | | |
| S5580–39 | 66 | 33 | 12 | 9 | 120 | 47.7 ± 6.4 |
| S5582–16 | 50 | 33 | 25 | 10 | 118 | 55.2 ± 7.4 |
| S5582–36 | 70 | 17 | 12 | 10 | 109 | 33.9 ± 5.8 |
| Joint estimate (excluding S5582–36 in 1960) | 356 | 167 | 130 | 63 | 716 | 49.1 ± 2.1 |

* Parental classes starred.

TABLE 5

*Segregations obtained in the third backcross,* barbadense *to* hirsutum *involving recombination in the* $yg_2$–$r_2$ *region*

| Families | $Yg_2R_2$ * | $Yg_2r_2$ | $yg_2R_2$ | $yg_2r_2$ * | Total |
|---|---|---|---|---|---|
| S5579 | 13 | 2 | 0 | 9 | 24 |
| S5580 | 26 | 2 | 4 | 20 | 52 |
| S5581 | 22 | 3 | 0 | 31 | 56 |
| S5583 | 16 | 1 | 2 | 19 | 38 |
| Total | 77 | 8 | 6 | 79 | 170 |

$\chi^2(9) = 10.6$; P = 0.30–0.50
C.O. percent = 8.2 ± 2.1

| | Individual gene segregations | | | |
|---|---|---|---|---|
| | | Total | $\chi^2(1)$ | P |
| Expected | 85:85 | 170 | . . | . . |
| $Yg_2$:$yg_2$ | 85:85 | 170 | 0.0 | 1.00 |
| $R_2$:$r_2$ | 83:87 | 170 | 0.1 | 0.70–0.80 |

* Parental classes starred.

TABLE 6

*Segregations obtained in the selfed progenies of three selections from third backcross families* (barbadense *to* hirsutum) *involving recombination in the* "$Cl_2$"—$yg_2$—$r_2$ *region. Dominant alleles (including* $cl_2$ *which shows reversed dominance in* hirsutum *are indicated by* (+), *recessive alleles by* (−)

| | | * | * | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $cl_2$ | + | − | + | − | + | − | + | − | |
| | $Yg_2$ | + | − | + | − | − | + | − | + | |
| Family | $R_2$ | + | − | − | + | − | + | + | − | Total |
| S5582–7 | | 46 | 26 | 38 | 3 | 0 | 2 | 0 | 1 | 116 |
| S5582–16 | | 75 | 18 | 21 | 2 | 1 | 0 | 1 | 0 | 118 |
| S5582–38 | | 50 | 19 | 11 | 1 | 1 | 2 | 0 | 1 | 85 |
| Total | | 171 | 63 | 70 | 6 | 2 | 4 | 1 | 2 | 319 |

$\chi^2(14) = 25.8$; P = 0.02–0.05

Components of heterogeneity

| Source | d.f. | $\chi^2$ | P |
|---|---|---|---|
| $cl_2$:"$Cl_2$" | 2 | 4.4 | 0.10 –0.20 |
| $Yg_2$:$yg_2$ | 2 | 1.6 | 0.30 –0.50 |
| $R_2$:$r_2$ | 2 | 13.1 | 0.001–0.01 |
| Linkages | 8 | 6.7 | 0.50 –0.70 |
| Total | 14 | 25.8 | 0.02 –0.05 |

* Parental classes starred.

TABLE 7

*Analyses of linked segregations involving unstable* $R_2$ *classes. Data from Table 6. The adjusted frequencies are given in parenthesis below each class. The adjustment factor,* d, *is estimated separately from each family as the proportion of* $R_2$ *types misclassified as* $r_2$. *For further information see text*

| Family | "d" | * $Yg_2R_2$ | $Yg_2r_2$ | $yg_2R_2$ | * $yg_2r_2$ | Total | C.O. percent |
|---|---|---|---|---|---|---|---|
| S5582–7 | 0.414 | 48 | 39 | 3 | 26 | 116 | ..... |
| | | (81.90) | (5.10) | (5.10) | (23.90) | ... | 9.2 ± 2.8 |
| S5582–16 | 0.119 | 75 | 21 | 3 | 19 | 118 | ..... |
| | | (82.69) | (5.81) | (5.81) | (23.69) | ... | 10.4 ± 3.0 |
| S5582–38 | 0.169 | 52 | 12 | 1 | 20 | 85 | ..... |
| | | (62.50) | (1.25) | (1.25) | (20.00) | .. | 3.0 ± 1.9 |
| Total (unadjusted) | | 175 | 72 | 7 | 65 | 319 | ..... |
| (adjusted) | | (227.09) | (12.16) | (12.16) | (67.59) | | |

Linkage heterogeneity between families: $\chi^2(2) = 3.8$; P = 0.10–0.20.

* Parental classes starred.

The data in Table 6 show that the three families gave heterogeneous segregations, and that the heterogeneity was almost entirely attributable to differences in the relative proportions of $R_2$ types recovered. It seems evident that despite the intensive scoring system adopted, a number of $R_2$ types had mutated prior to flowering and had, therefore, been misclassified as $r_2$. (As shown in Table 8, there

TABLE 7a

*Analyses of linked segregations involving unstable* $R_2$ *classes. Data from Table 6. The adjusted frequencies are given in parenthesis below each class. The adjustment factor,* d, *is estimated separately from each family as the proportion of* $R_2$ *types misclassified as* $r_2$. *For further information see text*

| Family | "d" | $cl_2 R_2$* | $cl_2 r_2$ | "$Cl_2$" $R_2$ | "$Cl_2$" $r_2$* | Total | C.O. percent |
|---|---|---|---|---|---|---|---|
| S5582–7 | 0.414 | 46 | 38 | 5 | 27 | 116 | ..... |
| | | (79.75) | (7.45) | (7.45) | (21.55) | ... | $13.8 \pm 3.5$ |
| S5582–16 | 0.119 | 76 | 22 | 2 | 18 | 118 | ..... |
| | | (83.81) | (4.69) | (4.69) | (24.81) | ... | $8.3 \pm 2.7$ |
| S5582–38 | 0.169 | 50 | 12 | 3 | 20 | 85 | ..... |
| | | (60.69) | (3.06) | (3.06) | (18.19) | .. | $7.5 \pm 3.0$ |
| Total (unadjusted) | | 172 | 72 | 10 | 65 | 319 | ..... |
| (adjusted) | | (224.05) | (15.20) | (15.20) | (64.55) | | |

Linkage heterogeneity between families: $\chi^2(2) = 2.4$; $P = 0.30–0.50$.

* Parental classes starred.

TABLE 8

*Analyses of individual gene segregations and linkage in the* "$Cl_2$"—$yg_2$—$r_2$ *region. Data from Tables 6 and 7. Adjusted classes in parenthesis*

| Individual gene segregations | | Total | $\chi^2(1)$ | P |
|---|---|---|---|---|
| Expected (3:1) | 239.25:79.75 | 319 | ... | ..... |
| $cl_2$:"$Cl_2$" | 244 :75 | 319 | 0.4 | 0.50 –0.70 |
| $Yg_2$:$yg_2$ | 247 :72 | 319 | 1.0 | 0.30 –0.50 |
| $R_2$:$r_2$ | | | | |
| S5582–7 | 51 :65 | 116 | 59.6 | 0.001 |
| S5582–16 | 78 :40 | 118 | 5.0 | 0.02 –0.05 |
| S5582–38 | 53 :32 | 85 | 7.3 | 0.001–0.01 |

Linkages

| $cl_2 Yg_2$* | $cl_2 yg_2$ | "$Cl_2$" $Yg_2$ | "$Cl_2$" $yg_2$* | Total | C.O. percent |
|---|---|---|---|---|---|
| 241 | 3 | 6 | 69 | 319 | $3.0 \pm 0.9$ |
| $Yg_2 R_2$* | $Yg_2 r_2$ | $yg_2 R_2$ | $yg_2 r_2$* | Total | |
| (227.09) | (12.16) | (12.16) | (67.59) | 319 | $7.9 \pm 1.6$ |
| $cl_2 R_2$* | $cl_2 r_2$ | "$Cl_2$" $R_2$ | "$Cl_2$" $r_2$* | Total | |
| (224.05) | (15.20) | (15.20) | (64.55) | 319 | $10.0 \pm 1.8$ |

* Parental classes starred.

was significant deficiency of $R_2$ types in all three families.) If one makes the assumption that early somatic loss of spot is independent of recombination in the segment, "$Cl_2$"—$yg_2$—$r_2$, during the preceding meiosis, then the effects of misclassification may be corrected in the following manner:

Let $p$ be the recombination fraction in the $yg_2$—$r_2$ interval. The expected proportions of the classes, $Yg_2\ R_2$, $Yg_2\ r_2$, $yg_2\ R_2$ and $yg_2\ r_2$ will be respectively,

$$\frac{2+P}{4}, \frac{1-P}{4}, \frac{1-P}{4}, \frac{P}{4}$$

where $P = (1-p)^2$

Let $d$ be the proportion of $R_2$ types misclassified as $r_2$. Then the expected proportions of the four classes will become, respectively,

$$\frac{(2+P)(1-d)}{4}, \frac{1-P+d(2+P)}{4}, \frac{(1-P)(1-d)}{4}, \frac{P+d(1-P)}{4}$$

If $e$ is the expected number of $R_2$ segregates in the family and $o$ the number actually scored as $R_2$ then $d = \frac{e-o}{e}$ and can be estimated separately for each family.

The recombination fraction, $p$, can then be estimated using a modified form of the recombination method.

$$\frac{Yg_2R_2 \times yg_2r_2}{Yg_2r_2 \times yg_2R_2} = \frac{(2+P)(1-d)(P+d(1-P))}{(1-P+d(2+P))(1-P)(1-d)} =$$

$$\frac{(1-d)\ P^2 + (2-d)\ P + 2d}{(1-d)\ P^2 - (2+d)\ P + (1+2d)}$$

Using this method, the crossover percentages between $Yg_2$ and $R_2$ and between $cl_2$ and $R_2$ were calculated and are shown in Tables 7 and 7a respectively. Although the crossover estimates derived from the different families have a rather wide spread, they are not significantly different. This can be shown by recalculating the expected segregations for each family from its appropriate estimate of $p$, and so obtaining the adjusted frequencies which are shown in parenthesis in Tables 7 and 7a. Contingency tests can then be conducted on the adjusted frequencies. Since in fitting the data, two degrees of freedom are lost in the case of each individual gene segregation, the chi-squared value associated with the remaining two degrees of freedom is attributable solely to heterogeneity in the linkage component. In neither Table 7 nor 7a is this component significant, so that the adjusted frequencies of the individual families can be combined to provide estimates of crossover frequency. These are shown in the lower portion of Table 8.

The crossover estimates in Table 8 indicate that the map sequence is "$Cl_2$"—$yg_2$—$r_2$ which agrees with the sequence $Lc_1$—"$Cl_2$"—$yg_2$ obtained previously from the data in Table 2. The interval, "$Cl_2$"—$yg_2$ is common to both sequences, and the crossover estimates of 4.5 percent in Table 2 and 3.0 percent in Table 8 are in good agreement. Since the estimate obtained in Table 2 is based on five families and that in Table 8 on only a portion of the data from three of the same five families, the value of 4.5 percent may be regarded as the more reliable

estimate. The interval, $yg_2$—$r_2$, furnishes an estimate of 7.9 percent in good agreement with the backcross estimate of 8.2 percent in the previous generation (Table 5).

*Principles of relative mapping:* If two chromosomes from related species are strictly homologous, then, under comparable conditions, their patterns of recombination in hybrid condition should be exactly the same as those resulting from intraspecific recombination. Comparable conditions imply (1) a common cytoplasm (2) a similar genotypic background and (3) similar environmental conditions (temperature, nutrition, etc.) particularly during the meiotic process. If, under such conditions, patterns of interspecific and intraspecific recombination can be shown to be significantly different, the natural conclusion to be drawn is that homology between the chromosomes is imperfect.

Differences in recombinational patterns can be measured by relative mapping. Homologous chromosomes from the same species and heterozygous in a sequence of marker loci are testcrossed to provide data on which a standard linkage map may be constructed. A parallel testcross is performed involving supposedly homologous chromosomes from related species and marked as far as possible by the same loci. This yields a second or relative map which can be compared region by region with the standard map. Since from the early classical work with Drosophila it is known that recombination is reduced in non-homologous regions, and that in corn (unlike Drosophila) there is a concomitant increase in neighboring regions (MORGAN 1950; RUSSELL and BURNHAM 1950; RHOADES and DEMPSEY 1953), a systematic comparison of standard and relative maps should indicate rather precisely the location of any non-homologous segment. At the same time, a comparison of total map length enables a distinction to be made between an over-all reduction in recombination and a shift in crossover distribution.

The data on interspecific recombination reported here are comparable with data on intraspecific recombination published earlier (STEPHENS 1955). Both sets of data involve the same chromosomal region, *N* through $r_2$. In both cases the cytoplasmic background is that of the *hirsutum* marker stock, SM 4, since the SM 8 stock used in the present studies was synthesized from an intra-*hirsutum* cross which employed SM 4 as female parent. In the case of the intraspecific data, the genotypic background is pure *hirsutum*, as far as is known. All the markers used originated as mutants in standard *hirsutum* (upland) varieties. In the case of the interspecific data (a third backcross of *barbadense* to *hirsutum*) the genotypic background should average over 90 percent *hirsutum*. Minor differences in genotypic background do not have an important effect on recombination (RHYNE 1958). Finally, although the two series of data were collected in different seasons, the crosses and selfings were made in the same greenhouse during the winter months when attempts are made to preserve standardized growing conditions from season to season. While the effects of fluctuating temperature, nutrition, etc., cannot be discounted, it is unlikely from the practical point of view that these would differ more between seasons under winter green-

house conditions, than between plants growing in the same season under field conditions.

The data to be compared are shown in Table 9. Where backcross and $F_2$ seg-

TABLE 9

*Relative maps of the* N—$Lc_1$—$yg_2$—$r_2$ *region as determined from recombination between (A) homologous chromosomes of* hirsutum *and (B) supposedly homologous chromosomes of* hirsutum *and* barbadense. *Intra-*hirsutum *estimates calculated from data of* STEPHENS *(1955)*

| Map interval | (A) Intra-*hirsutum* Estimated from | No. | C.O. percent | (B) *hirsutum* × *barbadense* Estimated from | No. | C.O. percent |
|---|---|---|---|---|---|---|
| $N$—$Lc_1$ | Backcross | 312 | 43.9 ± 2.8 | $F_2$ (coupling) | 716 | 49.1 ± 2.1 |
| $Lc_1$—$yg_2$ | Joint (B.C. + $F_2$) | 169 | 33.2 ± 4.6 | $F_2$ (repulsion) | 1348 | 39.6 ± 2.3 |
| $yg_2$—$r_2$ | Joint (B.C. + $F_2$) | 303 | 20.7 ± 2.7 | Joint (B.C. + $F_2$) | 489 | 8.0 ± 1.3 |
| Total ($N$—$r_2$) | | | 97.8 ± 6.0 | | | 96.7 ± 3.4 |

regations were both available, joint estimates of recombination have been calculated by the maximum likelihood method. Because the $cl_2$ marker was not available in the intraspecific data, it has been ignored in the interspecific data also, and map distances presented for the interval $Lc_1$—$yg_2$ (see Table 2). A comparison of the two sets of data shows that in the interval, $yg_2$—$r_2$, recombination has been reduced from 20.7 percent in the standard map to 8.0 percent in the relative map—a reduction of over 50 percent. Conversely, there has been a smaller *increase* in the other intervals, $N$—$Lc_1$ and $Lc_1$—$yg_2$. It will be recalled (Figure 2) that recombination in the $yg_2$—$r_2$ interval involves crossing over between *barbadense* and *hirsutum* segments while in the interval, $N$—$Lc_1$, only *hirsutum* segments are concerned. Recombination has, therefore, been reduced in the heterospecific region and compensated by increased recombination in the homospecific region. As a consequence, recombination over the total map length remains virtually unchanged (97.8—98.7 percent). The most reasonable interpretation of these findings is that the *barbadense* and *hirsutum* chromosomes are not completely homologous in the $yg_2$—$r_2$ region and, as a consequence, recombination is blocked over a portion of this interval. Owing to a compensatory increase in recombination elsewhere, the total recombination over the chromosome as a whole remains unchanged. *If this situation proves to be a common one in interspecific hybrids, it would indicate that the standard methods of counting chiasma frequencies per bivalent may sometimes provide quite misleading information on chromosomal homologies.*

In Figure 1c the relative map of the chromosome has been drawn to scale. This differs from the data presented in Table 9 in the fact that information on the $cl_2$—$yg_2$ interval is included in the map. It can be seen by comparing the standard *barbadense* map (Figure 1b) with the relative map (Figure 1c) that the distance between $cl_2$ and $R_2$ is practically unchanged. Assuming the same relative positions of $cl_2$ and $yg_2$, this interval is much longer in the standard *hirsutum* map (Figure 1a). This indicates that most of the interval, $cl_2$—$R_2$, in the *barba-*

*dense* chromosome has a homologous segment in its *hirsutum* counterpart, but the latter contains an extra segment not currently mapped in *barbadense*. Otherwise, the relative map should be shorter than either standard map in this region. The nature of the extra segment is at present unknown—it could represent the insertion of a segment from another chromosome (translocation), a duplication, or an inversion including $R_2$ and an unmapped region distal to it.

The material studied so far has only permitted relative mapping to be carried out in the heterospecific $cl_2$—$R_2$ end of the chromosome under investigation. With the stocks now available, one should be able to apply a similar analytical method to the $N$—$Lc_1$ region and, possibly, through interference analysis to locate the centromere. (It is known from earlier studies (STEPHENS 1955) that interference phenomena can be detected over long intervals in this chromosome and may be determined more by an inherently low chiasma frequency than by physical map distance.)

## DISCUSSION

*Compensatory recombination:* The decrease in recombination observed in the $yg_2$—$r_2$ region was accompanied by increases over the remaining segments of the total mapped region of the chromosome. The net effect of these negatively correlated changes was to leave the total map length of the chromosome unaffected. This suggests that the total recombination in this particular chromosome is under genetic control and that a decrease in one region may be rather exactly compensated by an increase elsewhere. This apparent compensation might be considered fortuitous were it not for the fact that in corn the data of MORGAN (1950); RUSSELL and BURNHAM (1950); and RHOADES and DEMPSEY (1953) lend themselves to a similar interpretation. These investigators studied independently a situation in which recombination in a marked chromosomal region, with or without an included inversion, could be compared. Two inversions were paracentric, one pericentric, and the map length in which they were included varied from 16 to 42 crossover units. Within each specified region, recombination in inversion heterozygotes was virtually the same as that found in structural homozygotes. Suppression of effective crossing over within the inversion loops was almost exactly compensated by increased recombination in the regions *proximal* to the inversions. In the *distal* regions recombination remained unchanged. Compensation restricted to the proximal regions may be inferred also in the case of the cotton material because the chromosomal map indicates that the non-homologous region is terminal or subterminal in position.

These data as a whole support the hypothesis that total recombination frequency *per bivalent* may be under quite stringent genetic control, though the positions where recombinations occur may be quite labile and influenced by local structure. The concept of a genetically limited recombination frequency is not new (DARLINGTON 1939) and has been incorporated into current understanding of the evolution of breeding systems under natural selection (STEBBINS 1950; GRANT 1958). The concept is also in accordance with the conservatism in map

length which is exhibited by most genetic material which has been studied adequately. As far as I am aware no published map length, save that of the sex chromosome in *Habrobracon juglandis*, is significantly in excess of 150 crossover units. This indicates that over a wide range of material the modal number of recombinations is restricted within the narrow range of 1–3 per bivalent. A similar conclusion may be reached from the masses of data which have been published on cytological counts of chiasma frequency. Since the map lengths in different species are quite unrelated to physical length, there would seem to be no plausible mechanical significance for the restriction. That it is an optimum restriction, fixed by natural selection and genetically determined, would seem to be a logical assumption. As a corollary, one might expect that mechanisms are available for buffering it against the effects of structural aberrations. The intrachromosomal compensation suggested here may provide one such mechanism—the interchromosomal compensation in Drosophila, another (SCHULTZ and REDFIELD 1951). Possibly the two mechanisms are alternative and of different relative importance in different genetic systems.

Two further points of interest are worth comment. In the corn data, compensation appears to be achieved in the immediate neighborhood of the non-homologous region—in cotton the compensation is spread over the remainder of the mapped region. There is no reason to expect that the pattern of adjustment—if such it is—would be identical in two unrelated species, but judging by their map lengths the corn chromosomes (2 and 3) have a greater chiasma frequency than is found in the cotton chromosome. The second point of interest is that in the corn data, the compensatory mechanism apparently applies only to the homologous regions, i.e. the crossover estimates do not include the genetically ineffective recombinations *within* the inversion loops. Nor is there compensation *distal* to the inverted regions. This may indicate that the compensatory mechanism is blocked by a break in the normal chromatin sequence, just as interference phenomena may be blocked by the interposition of a centromere.

*Cryptic structural differentiation:* In 1950, it was suggested that cytological, genetic and breeding behavior of fertile interspecific hybrids in Gossypium was not sufficiently explained by HARLAND's former theory (1936) of multiple gene substitution and that cryptic structural differences had developed with speciation. Both these concepts have become outdated with time and what was once controversial should become a dead issue. In current terms the simplest form of "mutation" probably involves a structural rearrangement i.e. a change in the order of base pairs in a nucleotide sequence. Most likely all fundamental genetic changes are "structural" at the molecular level and "cryptic" under the light microscope. From the data presented here, however, it is clear that normal meiotic pairing resulting in fertile progeny is not a reliable criterion of chromosomal homology. *There is a level of chromosomal differentiation between species which can be detected only by special techniques (e.g. preferential pairing, relative mapping) but which may have quite important consequences in plant breeding.* For instance, it would be unrealistic for a plant breeder to assume that the progeny of a fertile interspecific hybrid would segregate and recombine as freely as

an intervarietal cross. Depending on the specific patterns of chromosomal differentiation in the parental species, shifts in chiasma position and frequency could generate an unexpected amount of recombinational variation in some parts of the genome and completely block recombination elsewhere. New techniques are needed to make efficient use of interspecific hybrids (STEPHENS 1961a,b).

The precise nature of the non-homologous segment which differentiates the "homologous" chromosomes of *barbadense* and *hirsutum* reported in this paper remains unknown. It is probably significant that the $R_s$ (spot) locus associated with the region, though stable in its own species, becomes somatically unstable in interspecific combination. It is possible that an inversion is involved and that the somatic instability may result from a breakage-fusion-bridge cycle similar to that found in corn by MCCLINTOCK (1941). This possibility is under investigation. A second interesting feature is the reversal of dominance at the $cl_s$ locus. In Gossypium as a whole, dominance reversal has only been found in interspecific crosses and in all cases studied the reversal is only manifested in mutant loci which are "species-specific" (i.e. the mutant occurs in one parental species and is unknown in the other). The common assumption that the species in which the mutant has never occurred carries a normal allele at the same locus is only an assumption and needs experimental verification.

## SUMMARY

Two supposedly homologous chromosomes of *Gossypium barbadense* L. and *G. hirsutum* L. are shown to differ by a non-homologous terminal segment. In the non-homologous region crossing over is reduced from 20 to eight percent, but the reduction is compensated by slight but general increases in other marked segments of the chromosome. As a result, total map length remains virtually unchanged. The compensatory mechanism appears to be similar to that found in corn, where the reduction of crossing over in inverted regions is accompanied by increases in the immediately proximal regions. Cotton differs from corn in the fact that "compensation" is absorbed over most of the chromosomal arm and is not restricted to the immediately proximal region. It is pointed out that somatic instability and reversal of dominance in neighboring loci appear to be associated in some way with the lack of homology.

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# THE EFFECT OF SINGLE GENE SUBSTITUTIONS ON RESISTANCE TO RADIATION IN MICE[1]

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STRAIN differences, indicating differences in genetic factors, in response to radiation damage were first reported by HENSHAW (1944), who found that more radiation was required to produce a given effect in $LAF_1$ hybrid mice than to produce the same effect in inbred C3H mice. The effects he studied included the lethal dose, hematology, and the histopathology of the lymphoid organs, bone marrow, and testes. Since then, numerous workers have demonstrated strain differences in various responses to radiation (RUGH 1953).

GRAHN and HAMILTON (1957) found differences in the median lethal dose ($LD_{50}$) of four strains of mice. GRAHN (1958) crossed the least and most resistant of the four strains and estimated the heritability of the $LD_{50}$ from data on $F_1$, $F_2$, and a few $F_3$ offspring. The estimate, approximately 50 percent, indicated that the difference between the strains crossed was genetic.

From these studies we may conclude that genetic differences at many loci, collectively, can cause differences in radioresistance. The question remains whether single loci can influence resistance sufficiently to be important. HANCE (1928) reported that if mice heterozygous for the albino gene, *c*, were exposed to X-rays with partial body shielding, the unshielded portions grew white hairs, while mice homozygous for the color gene, *C*, did not show this effect. CHASE (1949) could not show any increased effect in six *BbCc* mice he irradiated. STORER (unpublished) compared normal heterozygotes (*SeSe*) and short ear homozygotes (*sese*) in the strain SEC/1Gn, and found that, under daily doses of 100 roentgens of X-rays, the two genotypes of each sex survived for different periods. GRAHN (1958) thought that the albino segregants from his outcrosses of $F_1$ females to A/Jax males were less resistant than their colored littermates. Neither of these investigators tested sufficient numbers of mice for an adequate experiment.

The purpose of this paper is to report the results of comparing mice differing at a single loci with respect to their resistance to radiation. Five inbred strains, each segregating at single loci, were used for this purpose. Mice of each of the three genotypes at the segregating locus were compared in each stock. The criterion of radioresistance was the survival time under daily doses of X-rays.

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## MATERIALS AND METHODS

Five inbred stocks of mice, each segregating for two alleles at a single locus, were used in this study. Table 1 shows the strains used in this study. The "number of generations inbred" represents the number of generations of brother-sister mating with heterozygosity forced on the segregating locus by backcrossing, except in the case of strain C57BL/6J-$a^t$. The latter strain was maintained by repeated backcrossing of $a^ta$ individuals to *aa* individuals from the inbred strain C57BL/6J.

In strains SEA/Gn and SEC/1Gn, the short ear locus was segregating for the normal (*Se*) and short ear (*se*) alleles. *SeSe* and *Sese* animals are normal in phenotype; *sese* individuals are distinguished by the reduced size of the external ear (LYNCH 1921) due to a severe reduction in the cartilage of the ear. GREEN (1951) reviewed the known pleiotropic expressions of the *se* gene, including many skeletal and cartilaginous defects, as well as some other anatomical abnormalities and reduced body size, and showed that most of these can be related to a single primary effect of the gene in reducing the growth rate of cartilage in the *sese* embryo. Embryonic mortality seems to be greater among *sese* embryos than among their normal littermates, and while *sese* individuals may survive as well as *SeSe* or *Sese* under favorable environmental conditions, *sese* animals succumb more readily to stress (SNELL 1931).

Strain C57BL/6J-$a^t$ carried two alleles at the agouti locus, black-and-tan, $a^t$, and nonagouti black, *a*. DUNN (1928) first described the action of the $a^t$ gene; $a^ta^t$ and $a^ta$ mice are black dorsally, with orange or yellow ventral fur, while *aa* mice are black on back and belly. No pleiotropic action of these genes is known. It should be noted that since this strain was maintained by backcrossing $a^ta$ individuals to the inbred strain C57BL/6J, which is homozygous *aa*, the *a* gene in this strain occurs on a genetic background that has been adapted to it by the process of selection in the formation of the inbred strain. The $a^t$ allele is foreign to the background on which it appears in this strain.

The HRS strain was segregating at the hairless locus for the normal (*Hr*) and hairless (*hr*) alleles. *HrHr* and *Hrhr* animals are normal in phenotype; *hrhr* animals appear normal until the age of about 10–14 days, at which time they begin to lose their hair. By the age of 20–24 days, *hrhr* mice are completely naked. At about six weeks of age, an abortive regeneration of the hair may take place, after which the mouse remains hairless until death (BROOKE 1926). Hairless

TABLE 1

*Strains and segregating loci*

| Strain | Segregating locus | Number of generations inbred |
|---|---|---|
| SEA/Gn | *Se/se* | 50 |
| SEC/1Gn | *Se/se* | 50 |
| C57BL/6J-$a^t$ | $a^t/a$ | 12 (BC) |
| HRS/Ls | *Hr/hr* | 15 |
| 129/J | $c^{ch}/c$ | 21 |

mice eat voraciously. In older mice, the skin tends to thicken and wrinkle. The eyes and teeth are weak and the nails tend to be abnormal (DAVID 1930). Prenatal and early postnatal viability of *hrhr* individuals is apparently normal, but after the loss of hair, the hairless mouse is less viable than normal; as might be expected, they are especially susceptible to low temperatures. CREW and MIRSKAIA (1931) noted that since the establishment of their colony in 1925, selection and outcrossing had improved the viability of the hairless mice. About 50 percent of their hairless females proved infertile, and those that were fertile could not suckle their young, for the mammary glands were abnormal. In our HRS strain, however, as in the hairless mice studied by STEINBERG and FRASER (1946), hairless females seemed normally fertile, and were usually successful in suckling their young. It would appear that this pleiotropic effect of the *hr* gene has also been altered by selection.

The 129/J strain carried two alleles at the albino locus, chinchilla, $c^{ch}$ and albino, *c*. The chinchilla gene, described by FELDMAN (1922), dilutes yellow pigment to a faint cream or ivory, but does not affect black pigment. In this strain, which was homozygous for pink-eyed dilution (*pp*) and white-bellied agouti ($A^{w}A^{w}$), $c^{ch}c^{ch}$ animals were a light orange-brown on the dorsal side with white bellies. Heterozygous $c^{ch}c$ animals were cream or ivory colored dorsally; their bellies also were white. Animals homozygous for the albino gene (*cc*) were, of course, pure white albinos. No information exists on the relative vigor of these three genotypes; in our strain, none of the three appeared to have an obvious advantage in vigor.

In each strain except 129/J, one or two pairs of heterozygous sibs were obtained from the generation indicated in Table 1 and mated together. Offspring of the dominant genotype were test-mated to homozygous recessive sibs. Production of seven mice, all of dominant phenotype, from such a cross was considered sufficient grounds to conclude that the animal under test was homozygous dominant.

One homozygous dominant male was then mated to two homozygous dominant and one homozygous recessive females. Simultaneously, one homozygous recessive male was mated to one homozygous dominant and two homozygous recessive females. In this way, approximately equal numbers of mice of each genotype, homozygous dominant, heterozygous, and homozygous recessive, were produced. The eight animals required for each set of six matings as described above were as closely related as possible. In most cases, all were full sibs; in a few cases one animal was a double first cousin of the rest.

In the 129/J strain the testing procedure was unnecessary since the three genotypes $c^{ch}c^{ch}$, $c^{ch}c$, and *cc* were distinguishable. Several pairs of sibs were obtained; one of each pair was of the genotype $c^{ch}c$, the other *cc*. Matings within these sib pairs gave $c^{ch}c$ individuals which were mated together to produce all three genotypes for testing.

The offspring of the genotype test matings made in each strain, except 129/J, were irradiated beginning at an age of 33 days. Tests on these mice will be referred to as tests A. Mice from the sets of six matings were also exposed at the age of 33 days; tests on these mice will be referred to as tests B. Other mice from

these same matings, or from the $c^{ch}c \times c^{ch}c$ matings in strain 129/J, were exposed beginning at an age of 120 days; these are the tests C. In each strain and test group, approximately ten animals of each sex and genotype were used.

The source of radiation was a General Electric Maxitron 250 deep therapy X-ray machine, run at 250 kvp, 20 ma. The mice were exposed in plastic centrifuge tubes on a rotating table 24 inches from the X-ray tube. A filter of 1.0 mm Al, 0.5 mm Cu was used. Each mouse was exposed to 70r daily from the beginning of exposure until it died. The number of days till death was used as the criterion of radiation resistance.

Daily readings of the dosage rate were made with a thimble chamber exposed on the rotating table on every day when mice were exposed. Due to variability of the power source, the average dosage rate received by different mice varied from 0.9r per second to 1.4r per second, although each mouse received a standard 70r per day, within the two percent error of the dosimeter. The average dosage rate received by a mouse was negatively correlated with the survival time. A covariance analysis was used to remove effects of the dosage rate both within and between groups. The results in the tables of the following section are, therefore, adjusted for the regression of survival time on average dosage rate.

## RESULTS

The results of the tests are summarized in Tables 2 and 3. Table 2 gives means and standard errors of survival times in days, adjusted for regression on the average dose rate, for each genotype-sex combination in each of the three groups. In Table 3, the analyses of variance for each test group are summarized.

TABLE 2

*Mean survival times and standard errors of the means*

| Test | Sex | Gene dose* | SEA/Gn *Se/se* | SEC/1Gn *Se/se* | C57BL/6J-$a^t$ $a^t/a$ | HRS/Ls *Hr/hr* | 129/J $c^{ch}/c$ |
|---|---|---|---|---|---|---|---|
| (A) | Male | 1 | 19.7 ± 0.76 | 18.0 ± 0.88 | 24.8 ± 1.22 | 20.5 ± 1.00 | ..... |
| | | 2 | 18.6 ± 0.72 | 17.2 ± 1.05 | 21.7 ± 2.17 | 20.3 ± 1.17 | ..... |
| | Female | 1 | 20.7 ± 0.69 | 16.4 ± 1.23 | 22.9 ± 1.26 | 20.3 ± 0.80 | ..... |
| | | 2 | 19.5 ± 0.89 | 17.1 ± 0.46 | 22.2 ± 1.46 | 23.6 ± 0.75 | ..... |
| (B) | Male | 0 | ..... | 12.0 ± 0.63 | ..... | 16.1 ± 0.77 | ..... |
| | | 1 | 16.1 ± 0.67 | 13.5 ± 0.16 | ..... | ..... | ..... |
| | | 2 | 14.8 ± 0.85 | 13.8 ± 0.23 | ..... | 15.2 ± 1.08 | ..... |
| | Female | 0 | ..... | 13.1 ± 0.26 | ..... | 16.2 ± 0.50 | ..... |
| | | 1 | 14.8 ± 0.41 | 13.4 ± 0.34 | ..... | ..... | ..... |
| | | 2 | 16.6 ± 0.95 | 13.8 ± 0.21 | ..... | 15.5 ± 0.56 | ..... |
| (C) | Male | 0 | 24.2 ± 1.00 | 19.2 ± 1.40 | 37.4 ± 0.57 | 23.5 ± 1.84 | 29.2 ± 2.79 |
| | | 1 | 24.3 ± 1.40 | 19.2 ± 0.40 | 24.1 ± 1.07 | 20.6 ± 2.44 | 36.1 ± 3.51 |
| | | 2 | 17.3 ± 1.16 | 21.4 ± 1.71 | 39.9 ± 1.10 | 26.8 ± 1.78 | 36.0 ± 3.03 |
| | Female | 0 | 17.3 ± 0.89 | 18.6 ± 1.03 | 31.3 ± 1.00 | 17.5 ± 0.61 | 34.3 ± 2.53 |
| | | 1 | 19.8 ± 0.92 | 18.8 ± 0.87 | 18.9 ± 0.61 | 19.9 ± 1.00 | 36.9 ± 3.04 |
| | | 2 | 23.9 ± 1.23 | 18.2 ± 0.77 | 32.5 ± 1.10 | 18.3 ± 0.93 | 38.2 ± 1.13 |

* Gene dose refers to the number of recessive genes, or of *c* genes in strain 129/J.

TABLE 3

*Results of variance analysis*

| Test group | Source of variation | SEA/Gn d.f. | SEA/Gn MS | SEA/Gn F | SEC/1Gn d.f. | SEC/1Gn MS | SEC/1Gn F | C57BL/6J-$a^t$ d.f. | C57BL/6J-$a^t$ MS | C57BL/6J-$a^t$ F | HRS d.f. | HRS MS | HRS F | 129/J d.f. | 129/J MS | 129/J F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (A) | Genotypes | 1 | 14.05 | 2.23 | 1 | 0.87 | <1 | 1 | 39.14 | <1 | 1 | 28.74 | 2.89 | .. | .... | .. |
| | G × S Interaction | 1 | 0.01 | <1 | 1 | 6.68 | <1 | 1 | 13.33 | <1 | 1 | 33.87 | 3.00 | .. | .... | .. |
| | Error | 34 | 6.31 | | 42 | 13.46** | | 31 | 29.28 | | 36 | 9.93 | | .. | .... | .. |
| (B) | Genotypes | 1 | 0.30 | <1 | 2 | 3.96 | 1.97 | .. | .... | .. | 1 | 3.31 | <1 | .. | .... | .. |
| | G × S Interaction | 1 | 15.64 | 4.60* | 2 | 0.07 | <1 | .. | .... | .. | 1 | 0.15 | <1 | .. | .... | .. |
| | Error | 22 | 3.40 | | 134 | 2.01** | | .. | .... | .. | 24 | 3.93 | | .. | .... | .. |
| (C) | Genotypes (linear) | 1 | 0.23 | <1 | 1 | 87.16 | 7.42* | 1 | 102.49 | 11.64* | 1 | 15.64 | <1 | 1 | 1.10 | <1 |
| | Genotypes (nonlinear) | 1 | 22.89 | 1.11 | 1 | 78.57 | 6.69* | 1 | 1031.22 | 117.05* | 1 | 5.45 | <1 | 1 | 2.36 | <1 |
| | G (linear) × S | 1 | 279.58 | 13.51* | 1 | 96.57 | 8.22* | 1 | 4.99 | <1 | 1 | 1.32 | <1 | 1 | 145.83 | 1.36 |
| | G (nonlinear) × S | 1 | 43.24 | 2.09 | 1 | 84.15 | 7.16* | 1 | 4.16 | <1 | 1 | 99.20 | 4.36* | 1 | 19.43 | <1 |
| | Error | 48 | 20.70** | | 48 | 11.75** | | 48 | 8.81 | | 36 | 22.73** | | 60 | 107.38** | |

* F test significant at the 0.05 level.
** Within subclass error variances nonhomogeneous.

*SEA/Gn:* In this strain, no *SeSe* animals were available for test B, so that in both test A and test B, the comparison was between *Sese* and *sese* animals, only. No differences were observed in test A. In test B, however, *Sese* males were more resistant than *sese*, while *Sese* females were less resistant than *sese*. In the mature mice in test C, where all three genotypes were compared, male *SeSe* and *Sese* were about equally resistant, and both were more resistant than *sese*. In females, on the other hand, resistance increased in the order *SeSe*, *Sese*, and *sese*; the increase in resistance was almost linear with increasing dosage of the *se* gene. These effects appear to be real, despite the nonhomogeneity of the subclass error variances, which throws some doubt on the validity of the F test.

It is not too surprising to find that an effect dependent on sexual differentiation appears in animals 120 days old, and not in animals 33 days old. More surprising is the fact that the effect does appear in one set of animals 33 days old (test B). A new and presumably more adequate ration was introduced between tests A and B. Body weights taken prior to the first exposure to radiation reveal that test A animals weighed less than those of test B, and were gaining weight more rapidly. These observations suggest that, although the animals of test A and of test B were chronologically the same age, the test B animals were developmentally more mature, perhaps due to the improved nutrition. This hypothesis, if true, would suggest a simple explanation of the greater resemblance of test B animals to the adult animals of test C.

*SEC/1Gn:* In this strain, the segregation of the genes *Se* and *se* apparently did not affect the radioresistance of test A or B animals. In test C, *SeSe* and *Sese* males were approximately equal in resistance, while *sese* males were more resistant. *SeSe* and *Sese* females were of approximately the same resistance, but *sese* females were less resistant. Nonhomogeneity of the subclass error variances was noted in all three test groups.

Thus, in both strains segregating at the short-ear locus, differences between the genotypes were found in mature animals. However, the differences were diametrically opposed in the two strains. In SEA/Gn, *se* increased the resistance of females, and decreased that of males. In SEC/1Gn, *se* decreased the resistance of females and increased that of males.

*C57BL/6J*-$a^t$: In this strain, there were no animals available for test B, so that only tests A and C were run. In test A, no differences were found. In test C, however, *aa* animals were more resistant than $a^ta^t$. Heterozygous $a^ta$ animals were much less resistant than either homozygote.

The difference in resistance between the homozygotes might be interpreted as an adaptation effect; the gene *a* is native to the rest of the genotype, while $a^t$ is not. There is, however, no apparent general effect on vigor, since the body weights of the two homozygous genotypes, taken before radiation, did not differ.

The low resistance of the heterozygous $a^ta$ animals appears to be an example of negative heterosis. It should be noted, however, that the three genotypes were not compared contemporaneously. Most of the homozygous animals were tested before any heterozygotes were available for testing. Some of the apparent reduced resistance in the heterozygote may therefore be due to temporal environ-

mental changes. Again, the factors affecting resistance do not seem to have general effects on vigor, for heterozygotes weighed more than either homozygous genotype.

*HRS/Ls:* In this strain, no heterozygous (*Hrhr*) animals were available for test B; therefore, in this test, the two homozygous genotypes were compared. Tests A and B revealed no differences between genotypes. In test C, heterozygous males were less resistant than either homozygote, while heterozygous females were more resistant than either homozygous female genotype. Again, the within subclass error variances were nonhomogeneous. In this case, the males varied more within subclass than did the females. Testing the two sexes separately, the genotypic differences within sex were not significant in either sex. The validity of the F test in the combined analysis is called in question by the nonhomogeneity of error variances; in the separate analyses, since this is a result-guided analysis, the F test is again not valid. The possible difference in radiation sensitivity due to the *hr*-locus should be tested further.

*129/J:* Since no test matings were made in this strain, there was no test A. The strain was slow in breeding, and it was not possible to obtain enough animals for test B. Therefore, only test C was run on this stock. No differences between genotypes could be shown.

## DISCUSSION

The results indicate that a single gene locus, marked by its effects on color or body conformation of the mouse, may also affect resistance to radiation. The effect may take the form of an interaction between sex and genotype, the difference between genotypes in males being different from that in females. A gene recessive in its effect on the visible phenotype may be recessive or partially dominant in its effect on resistance. The type of gene action may also differ in the two sexes. The effect of these genes on radioresistance does not seem to be mediated via general effects on vigor; in general, differences in resistance do not seem to be related to differences in body weight.

This finding has potential value in the evaluation of mechanisms of radiation damage. The correlation of resistance effects of a given gene and other known effects of that gene may yield important clues to mechanisms affording protection from radiation damage. Means of preventing or curing somatic radiation damage in animals or in man might be suggested by such correlations.

Differences between genotypes are likely to be greater in mature mice than in weanlings. As a result, despite the fact that within-class error variances are likely to be nonhomogeneous in mature mice, these seem to be preferable in attempting to assess differences between genotypes. This fact is obviously related to the fact that many of the differences observed are dependent on sex differences.

These results may also throw some light on the question of which sex is more resistant to radiation. ABRAMS (1951) reported no sexual difference in mice less than 90 days old. In our work, sexual differences did not appear in 33 day old mice, but did in mice 120 days old. It may be that ABRAMS found no sexual difference because his mice were too young.

ELLINGER (1950) reported that testosterone decreased the resistance of male mice to X-rays. PATT, SWIFT, STRAUBE, TYREE and SMITH (1949) reported increased resistance in mice given estradiol benzoate. CHAPMAK (1955) found that males were less resistant than females. RUGH and CLUGSTON (1955) also found females more resistant than males, and presented evidence that resistance varied cyclically in females with changes in the level of estrogen production during the estrus cycle.

On the other hand, GRAHN, SACHER and HAMILTON (1954) found females less resistant than males in three strains of mice with different average resistances.

Our results also indicate a general superiority of males in this respect. However, in two cases a single gene change was sufficient to alter the rank of the sexes. It is not hard to imagine that the rank of the sexes may differ between strains differing at many loci if a change at a single locus can affect this character. The irradiation schedule may also be involved. GRAHN, SACHER and HAMILTON used a repetitive periodic exposure schedule; our studies were carried out with a similar schedule. The investigators reporting that females were superior to males used a single exposure, either to determine an $LD_{50}$ or to compare time of survival and percent survival after the single dose of irradiation.

## SUMMARY

The results of irradiating animals differing at a single locus in each of five inbred strains are presented. Substituting the short ear gene (*se*) for its normal allele (*Se*) in two strains affected radiation resistance, the nature of the effect depending on sex and on the genetic background. Radiation resistance was also affected when the black-and-tan gene ($a^t$) was substituted for its nonagouti allele ($a$) in the C57BL/6J genotype. When the hairless gene (*hr*) was substituted for its normal allele (*Hr*) the results were not clear, but this substitution may have an effect. There was no indication of an effect from substituting the chinchilla gene ($c^{ch}$) for the albino gene ($c$).

The radiation resistance effects were obtained in adults; in general, younger mice did not show any effect. Sex is important in determining the effect of a given substitution on radiation resistance.

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# X-IRRADIATION OF THE DEVELOPING MALE GERM CELLS OF TRIBOLIUM CONFUSUM[1]

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IN radiobiological experiments the two primary variables are the source of radiation and the organism irradiated, and the observed effects will usually be altered when either one or both variables are changed. A comparison of the altered effects leads to a more precise understanding of the events which ensue when radiant energy is absorbed by a living system, and for this reason it is important to have available information obtained from a variety of radiation sources as well as from a number of different organisms. At the present time a considerable portion of the information concerning the genetic effects of radiation has been derived from studies involving the mouse and various species of Drosophila, but these organisms are so different from each other that the data obtained may not always lend itself readily to comparison. The flour beetles, *Tribolium confusum* and *castaneum*, on the other hand, might prove to be useful in this respect since in many of their biological attributes, such as size and reproductive characteristics, they are similar to Drosophila, whereas in other ways, for example, chromosome number and life span, they are quite different.

There have been, however, only occasional studies made of the effects of radiations on Tribolium. About 40 years ago, DAVEY (1919) reported that small doses of X-rays seemed to increase slightly the life span of *T. confusum*. More recently CORK (1957) measured the changes in the longevity of *T. confusum* in response to different doses of gamma radiation, ROGERS and HILCHEY (1957) studied some of the responses of Tribolium to beta radiation, and PARK, DEBRUYN, and BOND (1958) compared the effects of X-rays on the productivity and longevity of males and females of *T. confusum* and *castaneum*. DUCOFF and WALBURG (1960) have attempted to evaluate the effectiveness of X-rays as a means for the production of phenocopies, and the depression of fertility in *T. confusum* males following exposure to fast neutrons was observed by McDONALD and LONG (1961).

Detailed studies of the differential radiosensitivity of male germ cells, such as have been made with Drosophila and mice (for example, see ALEXANDER, BERGENDAHL and BRITTAIN 1959; and BATEMAN 1958) have not yet been undertaken with Tribolium. The following report is an analysis of this effect of X-rays on *T. confusum* and an attempt to compare this response of the flour beetle with the same response in Drosophila.

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## METHODS AND RESULTS

The strain of *T. confusum* used here originated from several prefertilized females captured in 1955. To analyze the effects of radiation on male fertility, the "brood pattern" technique was employed, whereby the treated male is mated sequentially with a number of virgin females. The percent of inviable eggs produced by the females is a reflection of the fertility of the irradiated male. This method, however, requires preliminary information concerning the onset of sexual maturity in males, for the fertility of a young male is likely to be a function of age as well as exposure to radiation. Data bearing on this problem are presented in Table 1. Adult males whose ages from the time of eclosion ranged from less than one to nine days were each placed in vials with a virgin female about nine days of age. Each vial contained five grams of the standard flour-yeast medium used for Tribolium. After 24 hours, the male was removed, but the female remained in the vial for another three days and was then transferred to a second vial for an additional four days. Six weeks later the number of offspring in each vial was counted. Unless otherwise stated, all experiments were conducted at a temperature of 30°C and a relative humidity of 50–70 percent.

Obviously, under these conditions, males do not attain complete sexual maturity until about three days after eclosion. Only one female was fertilized in group B, and in group C where the males were between two and three days of age when put into the vial and three to four days old when removed, only half the females were productive. Variation in the precociousness of males is suggested by the fact that two females in group D and one in group E were unproductive too. Apparently, most males are mature by the fifth day of their adult life, for all females in groups F and G were productive. A further observation is that the mean number of offspring per fertile female in group C (24.2) is significantly lower ($t = 5.6$, $P < 0.01$) than the mean number (81.9) in group

TABLE 1

*The number of offspring produced by females mated with males of different ages*

| Group | Age of male in days | Vial number | Pair number | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| A | <1–2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 1–3 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 2–4 | 1 | 36 | 0 | 0 | 10 | 3 | 32 | 11 | 0 | 0 | 0 |
| | | 2 | 2 | 0 | 0 | 0 | 1 | 26 | 0 | 0 | 0 | 0 |
| D | 3–5 | 1 | 44 | 0 | 54 | 42 | 38 | 32 | 43 | 0 | 37 | 49 |
| | | 2 | 40 | 0 | 44 | 50 | 46 | 19 | 35 | 0 | 39 | 43 |
| E | 4–6 | 1 | 41 | 28 | 39 | 39 | 40 | 35 | 0 | 53 | 41 | 41 |
| | | 2 | 50 | 50 | 40 | 45 | 48 | 26 | 0 | 37 | 18 | 51 |
| F | 5–7 | 1 | 48 | 34 | 48 | 47 | 26 | 54 | 45 | 28 | 27 | 26 |
| | | 2 | 47 | 39 | 38 | 46 | 13 | 61 | 47 | 36 | 35 | 16 |
| G | 6–8 | 1 | 32 | 38 | 44 | 31 | 8 | 34 | 49 | 38 | 51 | 41 |
| | | 2 | 31 | 42 | 22 | 34 | 3 | 38 | 54 | 47 | 41 | 44 |

D. This means that a young male, even though able to inseminate a female, still cannot elicit her full reproductive response, but the cause of this has not been determined. As a result of these studies, in the experiments described below, males two to three days old were chosen for irradiation and then, to minimize the possibility of sperm accumulating in the male, were immediately placed with females.

Irradiation was accomplished through the use of a Pickett X-ray machine operated at 180 kv and 20 ma and containing 5 mm of Al added filtration. At a target to sample distance of 30 cm, the dose rate was approximately 145r/minute. Adult males confined in a small circular plastic container were irradiated, some for ten minutes (1450r) and others for 20 minutes (2900r).

Following irradiation, each male was placed in a five gram vial with a single female for two days, then transferred to a second female for another two days and this was repeated until each male had been paired with 12 different females over a period of 24 days. A sample of eggs, usually about 60, was obtained in two collections from each female over the eight days following the introduction of the male. The eggs were incubated in five gram vials and the number of adults obtained from the egg samples was recorded five to six weeks later. A control consisting of ten unirradiated males was handled in the same way except that only ten females were provided for each male. All females used were eight to ten days old. The data from these experiments are provided in Table 2.

In an effort to detect long term effects, five irradiated males (2900r) and five controls were kept for eight weeks following the time of irradiation and then placed individually with a female for four days. The number of adults obtained from eggs collected from these females was then determined. This data can also be found in Table 2.

TABLE 2

*The percentages of eggs which failed to reach the adult stage*

| | | Periods | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L | Eight weeks later |
| Control | | | | | | | | | | | | | | |
| Eggs | | 542 | 600 | 540 | 587 | 600 | 600 | 594 | 481 | 600 | 594 | ... | ... | 273 |
| Percent inviable | | 37 | 22 | 25 | 17 | 11 | 19 | 10 | 11 | 16 | 14 | ... | ... | 18 |
| 1450r | | | | | | | | | | | | | | |
| Eggs | | 578 | 600 | 540 | 593 | 568 | 463 | 600 | 536 | 600 | 587 | 600 | 540 | ... |
| Percent inviable | | 48 | 49 | 43 | 42 | 57 | 70 | 50 | 29 | 24 | 21 | 23 | 29 | ... |
| 2900r | | | | | | | | | | | | | | |
| | Collection | | | | | | | | | | | | | |
| Eggs | 1 | 258 | 300 | 314 | 263 | 295 | 300 | 300 | 230 | 290 | 261 | 297 | 285 | 267 |
| | 2 | 234 | 300 | 270 | 277 | 303 | 270 | 300 | 261 | 275 | 277 | 303 | 270 | ... |
| Percent inviable | 1 | 75 | 74 | 66 | 76 | 88 | 90 | 90 | 68 | 64 | 51 | 52 | 53 | 22 |
| | 2 | 84 | 68 | 64 | 72 | 87 | 89 | 88 | 75 | 63 | 47 | 54 | 54 | ... |

## DISCUSSION

The data in Table 2 and Figure 1 show that the percentage of inviable eggs

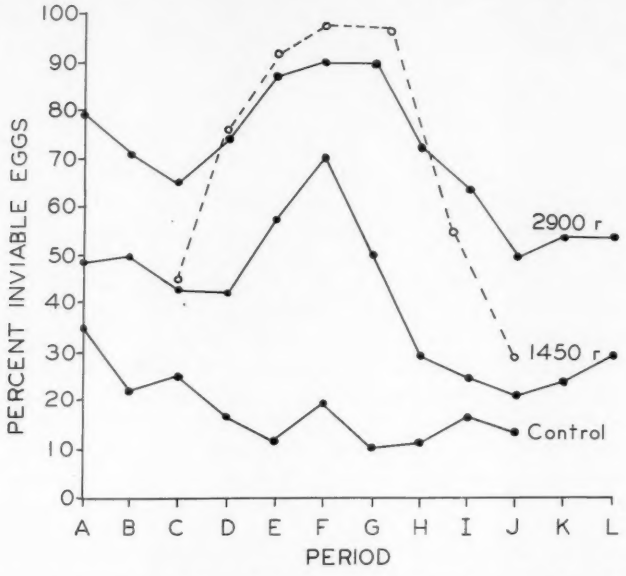


FIGURE 1.—A comparison of the radiation damage in the male germ cells of *T. confusum* (solid lines) and *D. virilis* (broken line). The Drosophila data was taken from ALEXANDER, BERGENDAHL and BRITTAIN (1959).

obtained from females inseminated by irradiated males reaches a maximum value at 11 to 12 days following exposure (period F) and declines gradually to a minimal value at about the 20th day (period J). A similar response is known to occur in other organisms, for example, in Drosophila. As SCHMID (1961) has recently pointed out, the phenomenon is by no means adequately understood; but differential sensitivity of cells in the various stages of spermatogenesis as shown by the induction of dominant lethal mutations, and the depletion of sperm supplies as a result of degeneration of damaged germ cells are considered to be of prime importance. Probably the same explanations apply with Tribolium, but fecundity measurements of irradiated males will be needed before each component's contribution to inviability can be determined. Furthermore, an additional factor may be involved in high values found for periods A and B. The control also shows higher percentages at first, and this fact coupled with the information concerning the onset of maturity in *T. confusum* makes the data of the first and perhaps second period of doubtful value as indicators of radiation damage. Nevertheless, the effects of the radiation are not entirely obscured even in period A, for the percentages in the control, the 1450r, and the 2900r samples,

are clearly different. However, in view of the above facts, it may be advisable in future experiments to age the males for several days in the presence of females before irradiating them.

Information concerning the temporal sequence of events in spermatogenesis of *T. confusum* is not yet available, so that the various stages of spermatogenesis cannot be assigned with certainty to the different periods shown in Figure 1. However, observations of Feulgen stained serial sections of the testes of one day old males have revealed the presence of all stages of spermatogenesis, including sperm, and since the irradiated males were at least two days of age when treated, it is certain that all the various cell types were exposed. If it is assumed, on the basis of evidence from other organisms, that early spermatids and meiotic cells are most sensitive to radiation, then period F and perhaps E and G include those cells, while periods A through D represent sperm and later spermatids, and H through L represent spermatogonia. Bearing in mind the assumptions involved, an attempt has been made to compare the response of *T. confusum* with that of *D. virilis* when irradiated with 2000r of 200 kv X-rays (Drosophila data taken from ALEXANDER, *et al.* 1959). In Figure 1, the highest percentage point of ALEXANDER's data (early spermatids) was made to coincide with the highest point of the Tribolium data (period F), and the other points were then plotted at appropriate distances on either side. The impression is gained that the responses of the two organisms are, in general, quite similar. There is some indication that the early spermatid and meiotic stages of *D. virilis* are more sensitive than the comparable stages of *T. confusum*, but definite conclusions must be deferred until more extensive Tribolium data become available.

The dosage-response curves plotted in Figure 2 suggest a departure from linearity within the periods H through L. This may be due in part to the high control level caused by the questionable values obtained for periods A, B, and C. However, even if these are eliminated and the control value is reduced as a consequence to about 14 percent, the dosage-response of cells in periods H through L still appear to be nonlinear. The average value for these periods at 1450r is about 11 percentage points over the reduced control, whereas the average value at 2900r exceeds the control by about 44 points. Thus, doubling the dosage approximately quadruples the damage in premeiotic cells. This information suggests a second difference between the response of Tribolium and Drosophila to X-rays, for ALEXANDER, BERGENDAHL and BRITTAIN (1959) found the production of dominant lethals in the premeiotic cells of *D. virilis* to be a linear function of dose.

It is possible that differential sensitivity is the explanation for the male fertility changes noted in *T. confusum* by PARK, DEBRUYN and BOND (1958). They found that the fertility of males receiving 2000 and 3000r of X-rays declined to a minimum at 12 to 15 days following treatment and then underwent a gradual increase. Since in their experiments the male remained with the same female continuously, and the female is able to store sperm, it would be necessary to assume either that the male inseminated the female only once when first placed with her, or that she was inseminated repeatedly but used each new batch of sperm preferentially. The latter explanation seems more likely, especially since

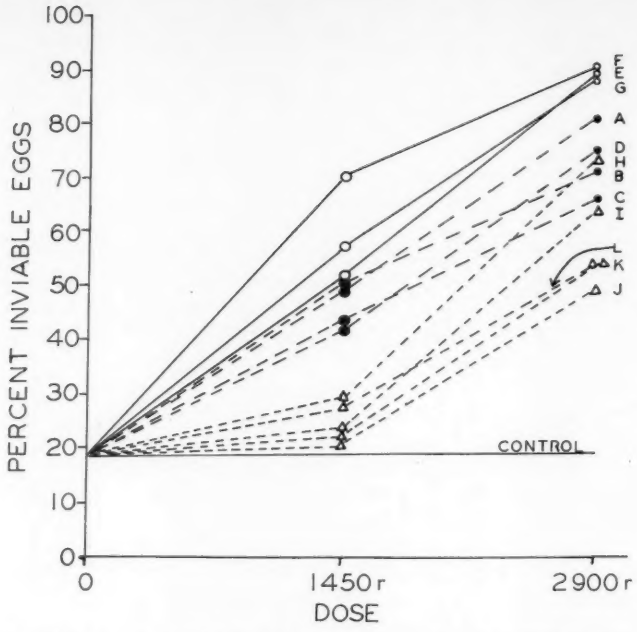


FIGURE 2.—The response of male germ cells to different doses of X-rays. Periods presumed to represent: mature sperm and late spermatids (●), early spermatids and meiotic cells (○), and premeiotic cells (△).

SCHLAGER (1960) has demonstrated the existence of sperm precedence in *T. castaneum*.

The data in Table 2 have been arranged so that the individual four-day egg collections and adult counts from every period appear separately. This provides an opportunity to explore the possibility that irradiated sperm may recover with time, for sperm fertilizing second collection eggs had been stored by the female during the preceding four days while sperm for first collection eggs were being utilized. Therefore, if sperm are able to recover, a higher percentage of viable eggs might be expected in second collection eggs than in those of the first collection. The evidence available here, however, gives no indication of recovery. Viability is not consistently better in either egg collection and, in fact, when all periods are combined, the proportion of viable and inviable eggs in the two collections are not significantly different ($x^2 = 0.28$, $P = 0.60$). The possibility of sperm recovery occurring, however, should not be abandoned until a more thorough investigation is made over a longer period of time. This should prove to be entirely practicable with Tribolium, for the female of *T. confusum* can obtain from a single insemination sufficient sperm to fertilize her eggs for more than three months.

Finally, there is no indication in Table 2 of any extended effect of the radiation, such as the sterile period found in mice (BATEMAN 1958), or the prolonged depression of fertility which occurs in Tribolium following higher dosages (PARK, DEBRUYN and BOND 1958). Although the percentage of inviable eggs obtained from females inseminated by males eight weeks after irradiation is slightly higher than the control percentage, this difference is not significant ($\chi^2 = 0.975$, $P = 0.32$).

## SUMMARY

Young adult males of *T. confusum* have been shown to require about four days, following eclosion, to reach sexual maturity. Subjecting two or three day old males to 1450 or 2900r of X-rays depresses their fertility, as evidenced by the increase in the percentage of inviable eggs produced by their mates. This inviability, probably due in part to the induction of dominant lethals, reaches a maximum value 11 to 12 days following irradiation and then declines. In general, the pattern of response suggests that the various stages of spermatogenesis of *T. confusum* are differentially sensitive to X-rays. No evidence was found for recovery of irradiated sperm stored by females, or of extended effects on the fertility of the treated males.

## ACKNOWLEDGMENTS

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# THE VARIABILITY OF RESPONSE TO SELECTION I. INTERLINE AND INTRALINE VARIABILITY IN A POPULATION OF DROSOPHILA AFFINIS SELECTED FOR BODY WEIGHT[1]

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MOST expectations relating to breeding systems have been derived under the assumption of an infinite or very large population and are based almost entirely upon deterministic theories in which random variation is discounted (KEMPTHORNE 1957). However, the advance of a finite genetic population through time is subject to variation through random sampling as a consequence of Mendelian segregation and selection. Therefore, quantitative estimates of the magnitude of the random variation inherent in finite breeding systems are necessary in order to determine the reliability of the existing expectations.

Results from selection experiments involving both large animals and laboratory organisms have demonstrated that selection is a powerful tool for changing the genetic composition of a population. The expected rate of change per generation may be estimated from population genetic theory if the selection differential and the heritability of the characteristic under consideration are known. Actual responses, however, may be quite variable when only a small effective number of parents is used to perpetuate selected lines if random sampling is an important source of variation in a selection program.

The variability of response to selection has apparently not been thoroughly studied and the various experiments designed to test this variability are not in complete agreement (CLAYTON, MORRIS and ROBERTSON 1957; MARIEN 1958; RASMUSON 1955; ROBERTSON 1955). Quantitative estimates of the variability between lines selected alike are needed to determine if the variability in response to selection is an important source of variation in a selection program. The primary objective of the present study is to obtain the interline and intraline variance components from replicated lines of *Drosophila affinis* subjected to two intensities of selection for body weight in each of two directions. With such information, it shall be possible to construct intervals to attach to various means in order to indicate the magnitude of the variability of response to selection.

## MATERIALS AND METHODS

The body weight of Drosophila is easily measured with the proper facilities and has responded to two-way selection in a large population at a low degree of

[1] The data in this paper are from a thesis submitted by the senior author to the Graduate College of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy, February, 1961.

inbreeding (MARTIN 1959; MARTIN and BELL 1958, 1960). When selected lines are perpetuated by a small effective number of parents, it is feasible to incorporate much replication into an experimental design using Drosophila. Therefore, the body weight of Drosophila was chosen to be studied in this selection experiment.

The stock used in this study was derived from a large population that was trapped in Urbana, Illinois, in 1959 and later identified as *Drosophila affinis* Sturtevant (STURTEVANT, personal communication). This population was random mated for 14 generations in the laboratory before selection was initiated in order to insure that the population was in or near a state of genetic equilibrium. The laboratory in which the study was conducted is air-conditioned and the mean daily temperature is maintained at approximately 76°F.

All flies from which data were taken were reared in 100 × 23 mm shell vials containing approximately one half inch of the usual corn meal-karo medium which had been slanted and then lightly dusted with live yeast. Parents were removed from the vials when pupation began. The flies were weighed individually to the nearest microgram on a microbalance which was found to yield highly repeatable results. The intrasex repeatability, as determined by three repeated individual weighings of 25 males and 25 females, was found to be 0.96. All flies were weighed between zero and 14 hours after emergence, and most within 12 hours, in order to reduce the effect of ovarian development.

## EXPERIMENTAL RESULTS

*Parameter estimation:* Three large samples of data were obtained from individuals representing generations 12, 13 and 19 of random mating. The mean body weights of males and females and the average of these means were determined from the samples and these are presented in Table 1. The data of generations 12 and 13 were collected during February and March of 1960, whereas those of generation 19 were obtained during June of the same year. These data confirm the general observation that the body size of the flies was smaller during the summer months, even though the room temperature in the laboratory was maintained by a thermostatically controlled air conditioner. From this table it is seen that the average body weight of *Drosophila affinis* reared under the culturing conditions of this laboratory is approximately 850 micrograms, the average weight of females exceeding that of males by approximately 200 micrograms.

TABLE 1

*Mean body weight of* Drosophila affinis *in micrograms*

| | Generation of random mating | | | | | |
|---|---|---|---|---|---|---|
| | 12 | | 13 | | 19 | |
| Sex | Mean | n | Mean | n | Mean | n |
| Male | 785 | 400 | 751 | 415 | 694 | 1019 |
| Female | 949 | 400 | 946 | 415 | 891 | 1024 |
| Average | 867 | 800 | 849 | 830 | 793 | 2043 |

The phenotypic variance of body weight of males and females was calculated individually from data of each of the three samples and these estimates are presented in Table 2. The magnitude of the variance ratios indicates a highly significant difference between the variances of the sexes in each sample. The remarkable similarity among estimates of the variance in body weight of males should be noted.

Pooled regressions of the average body weight of five male and five female offspring on the individual weights of male and female parents, respectively, indicated that the heritability of body weight in *Drosophila affinis* is approximately 0.06, the heritability associated with the male parent being somewhat higher than that of the female parent, 0.08 *vs.* 0.04. Full-sib correlations indicated that the heritability of body weight was substantially higher, approximately 0.50, but this estimate is believed to be greatly inflated because of the environmental correlation common to full-sibs reared in the same vial. Paternal half-sib correlations were also determined, but these were found to be negative. It is possible that these negative paternal half-sib correlations, as well as the higher heritability which is associated with the male parent, may be due in part to the presence of a negative maternal effect on body weight in Drosophila which is described elsewhere( DeFries and Touchberry 1961).

*Selection experiment:* Ten vials representing generation 14 of random mating were randomly assigned the numbers one through ten. From each of these ten vials, ten males and ten females were randomly drawn and individually weighed between zero and 12 hours after emergence. The heaviest male and female within each vial were mated and the resulting single pair matings were designated lines 1–10, respectively. The lightest pair of the ten from each vial was also mated and these matings were designated lines 11–20. From each of the same ten vials, a second random group was drawn, but this time consisting of four males and four females. As before, the heaviest and lightest pairs were mated and the resulting lines denoted 21–30 and 31–40, respectively. Finally, one pair from each of the original ten vials was randomly chosen and mated, yielding lines 41–50. The direction and intensity of selection applied to each of the groups of lines may be summarized as follows: lines 1–10, upward at ten percent (U10%); lines 11–20, downward at ten percent (D10%); lines 21–30, upward at 25 percent (U25%); lines 31–40, downward at 25 percent (D25%); and lines 41–50, unselected (100%).

TABLE 2

*Phenotypic variance of body weight in* Drosophila affinis

| Sex | Generation of random mating 12 Variance | n | 13 Variance | n | 19 Variance | n |
|---|---|---|---|---|---|---|
| Male | 9,176 | 400 | 9,246 | 415 | 9,274 | 1019 |
| Female | 18,636 | 400 | 22,197 | 415 | 17,997 | 1024 |
| Variance ratio | 2.0** | | 2.4** | | 1.9** | |

** Significant at the 0.01 level of probability.

The progeny of these matings were designated the first selected generation. It was intended to select each line each generation as intensively as the parents of the first generation had been selected in that particular line. Thus, selection was to be practiced at two intensities in each of two directions, plus a control group, with ten lines in each of the five selection groups. This design would permit the experimental determination of the interline and intraline variability in response to selection even if a number of lines were lost. Occasionally, provisional matings had to be used when the selected parents produced no offspring. When this happened, it was necessary to compensate for the lowered selection intensity by selecting more intensively in the succeeding generations. A table of selection differentials in terms of standard deviations attained by various intensities of selection was consulted to determine how intensively one should select in later generations to compensate for the previous loss.

It should be noted that the parents of all 50 lines were selected contemporaneously from only ten full-sib groups, with all five selection "intensities" being applied to each. For example, lines 1, 11, 21, 31 and 41 were all selected differently but were derived from the same full-sib group. This design should tend to reduce any confounding of effects of the various selection intensities with the experimental material on which they were applied.

The amount of random variation caused by chance at Mendelian segregation in a selection program is probably greatest when the effective number of parents is small. The use of single pair matings to perpetuate the lines, i.e. the smallest possible effective number of parents, therefore, should maximize the amount of random variation caused by chance segregations. Since each line is closed, however, it is necessary to use full-sib mating, thus resulting in a high degree of inbreeding. Nevertheless, it was decided to use single pair matings because it made possible the handling of more lines. ROBERTSON (1955) has stated that the consistency of results in selection experiments is less when there is a high degree of inbreeding and a small foundation population. Therefore, this design should provide an estimate of the upper bound of the interline variability.

In order to measure the mean and variance of each selection group each generation, it was planned to weigh at least five male and five female offspring from each line, but sometimes less than five flies of each sex were available. Whenever more than five flies of a sex were weighed only the first five were included in establishing the mean and variance for that particular line and generation.

Because of the continual loss of lines throughout the experiment it was decided that the tenth generation of selection should be the terminal point for this study, before the data of that generation were analyzed. Only 18 of the original 50 lines had not been lost by the tenth generation and there were only two remaining lines in the D25% selection group.

In general, ten generations of selection were accompanied by a definite, but variable, response. There was a general decline in the mean body weight of the lines throughout the course of the study, except in the U10% group. Since full-sib mating was used throughout the study, it is possible that some of the general decline was the result of inbreeding depression; in the tenth generation of selec-

tion the coefficient of inbreeding was approximately 90 percent. However, because all lines were inbred at the same intensity and mated contemporaneously, any effects from changes in level of environment were confounded with those of intensity of inbreeding. The mean body weight of each selection group was adjusted for sex differences each generation and these adjusted means, as well as the number of lines present in each selection group, are presented in Table 3. It should be noted that the group means in the later generations are based on fewer observations than the earlier generations because of the continual loss of lines.

Because of the large variation between generations, the divergence between the high and the low groups of lines at the two intensities of selection was determined for each generation. This divergence is expressed on an intrageneration basis and is free of intergeneration differences in inbreeding and level of environment, except for any interaction which may have occurred between heredity and environment. The divergence between the high and the low selected groups, as well as the cumulative realized selection differential, is presented for each generation at the two intensities of selection in Table 4. In addition, the divergence between the high and low selection groups is plotted in Figure 1 and it may be seen that there is still much fluctuation between generations, with the large responses following the small ones. It is possible that this "rebounding effect" may be associated with the negative maternal effect which was mentioned previously.

The loss of lines throughout the selection experiment resulted in a confounding of the generation means with line differences. Therefore, a least squares method was employed to determine the generation means, independently of the lines, in order to measure any bias that might have arisen as a result of this confounding. A set of simultaneous equations was constructed involving line means containing both line constants and the group mean. The line constants were absorbed

TABLE 3

*Mean body weight of selection group of Drosophila in micrograms*

| Generation of selection | Selection group | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | U10% | *n** | D10% | *n* | U25% | *n* | D25% | *n* | 100% | *n* |
| 0 | ... | .. | ... | .. | ... | .. | ... | .. | 847 | 10 |
| 1 | 733 | 10 | 725 | 10 | 743 | 10 | 706 | 10 | 751 | 10 |
| 2 | 720 | 9 | 693 | 8 | 735 | 7 | 715 | 8 | 741 | 10 |
| 3 | 754 | 8 | 690 | 8 | 764 | 7 | 676 | 6 | 713 | 9 |
| 4 | 711 | 6 | 665 | 8 | 750 | 5 | 710 | 6 | 740 | 9 |
| 5 | 739 | 6 | 683 | 8 | 694 | 4 | 726 | 5 | 734 | 8 |
| 6 | 750 | 6 | 653 | 7 | 731 | 4 | 644 | 4 | 749 | 7 |
| 7 | 725 | 6 | 691 | 6 | 704 | 4 | 652 | 4 | 720 | 7 |
| 8 | 727 | 6 | 622 | 3 | 707 | 4 | 684 | 4 | 709 | 5 |
| 9 | 740 | 6 | 650 | 3 | 770 | 3 | 634 | 3 | 715 | 5 |
| 10 | 827 | 5 | 639 | 3 | 689 | 3 | 636 | 2 | 696 | 5 |

* *n* refers to the number of lines remaining in the respective selection group.

TABLE 4

*Divergence between high and low selected groups in micrograms*

| Generation of selection | 10% | | 25% | |
|---|---|---|---|---|
| | Response | Cumulative realized selection differential* | Response | Cumulative realized selection differential* |
| 0 | 0 | 0 | 0 | 0 |
| 1 | 8 | 230 | 37 | 140 |
| 2 | 27 | 442 | 20 | 232 |
| 3 | 64 | 559 | 88 | 358 |
| 4 | 46 | 762 | 40 | 515 |
| 5 | 56 | 869 | −32 | 645 |
| 6 | 97 | 1050 | 87 | 815 |
| 7 | 34 | 1246 | 52 | 919 |
| 8 | 105 | 1296 | 23 | 1013 |
| 9 | 90 | 1389 | 136 | 1201 |
| 10 | 188 | 1527 | 53 | 1480 |

* Corrected for line differences.

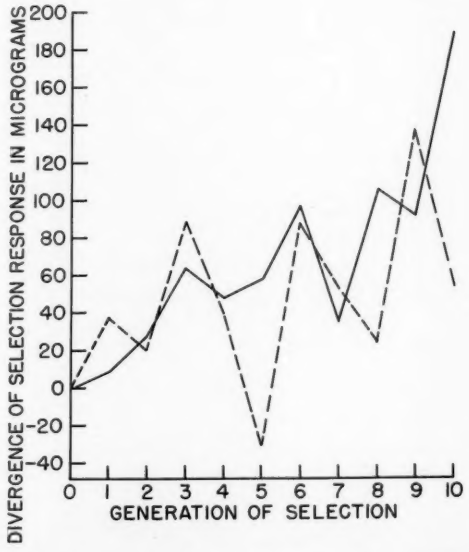


FIGURE 1.—Divergence between high and low groups at two intensities of selection. The solid line refers to the divergence at the ten percent intensity of selection, whereas the dashed line refers to the divergence at the 25 percent intensity of selection.

into the generation constants and the resulting equations were solved for the generation constants by an iterative method. The resulting generation constants were then substituted back into the original equations and the line means were evaluated. Generation constants were found for the high and low groups at both

intensities of selection. Also, the mean of each of these groups was obtained from the average of the line means. Thus, it was possible to determine the divergence between the high and low groups by adding the respective differences between the generation constants to those between the group means. This least squares method yielded similar results to those obtained from the actual differences between the high and low group means. Thus, it was concluded that there was little or no bias present in the previous results due to the confounding of the generation means with line differences. Consequently, the divergences of response presented in Table 4 are based on the actual differences between the means of the high and low groups.

The loss of lines also resulted in a confounding of the selection differentials with line differences. Thus, it was necessary to determine the cumulative selection differential of each group at a particular generation, independently of lines, and this was accomplished by calculating the cumulative selection differentials on a basis of only those lines which remained. The difference between the means of the parents of the high lines and those of the low lines which remained was found for all previous generations and the divergence of response in the previous generations for those lines was then subtracted from that difference, yielding the total cumulative selection differential at that particular intensity of selection. The cumulative selection differential was determined for each generation of selection at both intensities of selection in this manner. These differentials deviate somewhat from those in which line differences are included; thus, the cumulative selection differentials presented in Table 4 are those calculated independently of line differences.

The realized heritability was estimated from the regression of the divergence of response on the cumulative selection differential. The regressions, beginning with generation zero, from the data of the ten percent and 25 percent selection groups were 0.09 and 0.04, respectively. It should be noted that these realized heritability estimates are very close to those predicted from the pooled parent-offspring regressions previously presented. MARTIN (1959), using several different methods, has estimated that the heritability of body weight in *Drosophila melanogaster* is approximately 0.2.

It was questioned whether the intense inbreeding in this selection experiment may have reduced the selection differentials or the responses to selection. In order to check this possibility, regressions of the selection differentials (not cumulative) and the divergences of response (as measured by intergeneration differences) on $(1 - F)^{1/2}$ were calculated. $(1 - F)$ estimates the fraction of the additive genetic variance remaining within a given line at a given coefficient of inbreeding $(F)$. It was found that for each one percent increase in $(1 - F)^{1/2}$, the selection differential at the ten percent intensity increased by 1.8 micrograms, whereas that at the 25 percent intensity decreased 1.3 micrograms. Similarly, for each one percent change in $(1 - F)^{1/2}$, the response to selection at the ten percent intensity decreased 0.005 micrograms and that at the 25 percent intensity, increased 0.002 micrograms. None of the above regressions was found to be significantly different from zero. That there is no significant relationship between the coefficient of

inbreeding and these variables may be due to the fact that the additive fraction of the total variance of body weight in *Drosophila affinis* is relatively small.

*Interline and intraline variances:* The primary objective of this study was to measure the interline and intraline variability in response to selection. Components of variance between ($\sigma_b^2$) and within ($\sigma_w^2$) lines were estimated for each sex and for each of the five selection groups each generation. These components were calculated from the following relations:

$$\text{Expected mean square between lines} = \sigma_w^2 + n_o \sigma_b^2 \tag{1}$$

$$\text{Expected mean squares within lines} = \sigma_w^2 \tag{2}$$

$$n_o = \frac{1}{n_L - 1}\left[n. - \frac{\Sigma n_i^2}{n.}\right] \tag{3}$$

where $n_L$ is the number of lines, $n.$ is the total number of individuals of a given sex in the particular group under consideration and $n_i$ is the number of individuals of a given sex in the $i$th line of a group. The various components of variance were estimated by equating the calculated mean squares to the expected mean squares. A complete listing of the components for each generation would require an extensive table; therefore, only components from generations 1, 5 and 10 are contained in this presentation and these are found in Table 5. From this limited sample, however, the large variation between and within generations may be noted.

The interline and intraline variance components may be used to calculate

TABLE 5

*Variance components between and within lines of selection groups*

| Generation of selection | Selection group | Males | | Females | |
|---|---|---|---|---|---|
| | | Within | Between | Within | Between |
| 1 | U10% | 3,239 | 2,311 | 4,182 | 5,475 |
| | D10% | 3,171 | 2,317 | 4,637 | 814 |
| | U25% | 2,081 | 1,576 | 6,734 | 11,105 |
| | D25% | 2,795 | 1,684 | 5,825 | 7,530 |
| | 100% | 4,910 | 2,300 | 3,994 | 5,172 |
| 5 | U10% | 6,861 | 1,089 | 6,608 | 3,145 |
| | D10% | 5,374 | 6,039 | 5,115 | 6,519 |
| | U25% | 6,506 | — 533 | 18,796 | —3,526 |
| | D25% | 4,458 | 1,966 | 11,001 | 4,651 |
| | 100% | 6,584 | 3,263 | 7,073 | 5,643 |
| 10 | U10% | 9,108 | 3,887 | 12,978 | 11,045 |
| | D10% | 4,438 | 2,742 | 5,338 | 1,699 |
| | U25% | 13,364 | 5,278 | 9,916 | 11,487 |
| | D25% | 4,542 | 13,542 | 4,467 | 4,370 |
| | 100% | 3,486 | 5,282 | 7,861 | 10,879 |

confidence intervals for the mean of a particular line, means of lines selected alike and the mean of lines selected alike. The confidence intervals (CI) for the three criteria of grouping means are presented in equations 4–6, respectively.

$$\text{CI of the mean of a line} = \overline{Y} \pm [t_{a,(n_0-1)}]\left[\frac{\sigma_w^2}{n_0}\right]^{1/2} \quad (4)$$

$$\text{CI of the means of lines selected alike} = \overline{Y} \pm [t_{a,(n_0-1)}]\left[\frac{\sigma_w^2 + \sigma_b^2}{n_0}\right]^{1/2} \quad (5)$$

$$\text{CI of the mean of lines selected alike} = \overline{Y} \pm [t_{a,(n_0-1)(n_L-1)}]\left[\frac{\sigma_w^2}{n_0 n_L} + \frac{\sigma_b^2}{n_L}\right]^{1/2} \quad (6)$$

where $\overline{Y}$ is the appropriate mean and "$t$" is the appropriate value from the t-distribution, given the number of degrees of freedom and the probability of a Type I error ($a$), i.e. the probability of rejecting a true hypothesis. The level of probability to be used in the ensuing discussion will be 0.05; therefore, it should be expected that not more than 0.05 of the actual values will occur outside of the calculated intervals.

Equation 5 was used to calculate the confidence intervals for the means of lines selected alike and these intervals were attached to the mean body weight of selection groups; the resulting confidence intervals, pooled with respect to sex, are presented in Table 6. In these and the following intervals, $n_0$ was considered as being equal to five for each sex and $n_L$ was assumed to be equal to ten each generation. These intervals were compared to the actual means of lines selected alike and it was found that the mean of only one line in one generation, line 42 in the third generation, occurred outside of the calculated interval. It should be noted

TABLE 6

*Confidence intervals for the means of lines of Drosophila selected alike*

| Generation of selection | Selection group | | | | |
|---|---|---|---|---|---|
| | U10% | D10% | U25% | D25% | 100% |
| 0 | ..... | ..... | ..... | ..... | 728–967 |
| 1 | 576– 890 | 613– 836 | 547– 940 | 535–877 | 594–908 |
| 2 | 487– 953 | 566– 821 | 545– 925 | 626–804 | 584–898 |
| 3 | 512– 996 | 567– 814 | 618– 910 | 472–880 | 615–812 |
| 4 | 522– 900 | 549– 781 | 580– 920 | 492–928 | 596–884 |
| 5 | 603– 876 | 484– 881 | 633– 866 | 565–887 | 552–915 |
| 6 | 555– 944 | 517– 789 | 625– 837 | 430–857 | 534–965 |
| 7 | 472– 979 | 506– 860 | 517– 891 | 539–766 | 578–863 |
| 8 | 493– 960 | 387– 856 | 588– 827 | 509–860 | 526–892 |
| 9 | 582– 899 | 276–1024 | 558– 983 | 317–951 | 581–849 |
| 10 | 590–1064 | 504– 774 | 531–1009 | 406–865 | 475–918 |

that the estimates of the various components, and hence the intervals, are based on fewer observations in the latter generations.

From the magnitude of the intervals presented in Table 6, it is seen that the means of lines selected alike may vary considerably under the conditions of the present study. An analogy may be drawn between selection in a line of Drosophila perpetuated by only two parents and selection in a small herd of domestic animals which has only one sire in service at a time. If the variability in response to selection is as high or higher in domestic animals as it is in Drosophila, it may be seen from Table 6 that one could not be certain of much progress from selection in a small herd, even after ten generations. With the extremely small effective number per line and full-sib mating, chance changes in gene frequency are seemingly more important than those brought about by selection. Perhaps one of the greatest advantages of artificial insemination in dairy cattle is the availability of a large effective number of sires for small herds, thus reducing the large chance variation resulting when only one sire is used.

Equation 6 was used to calculate the confidence intervals for the mean of lines selected alike and these intervals are presented in Table 7. A comparison of Table 7 with Table 6 indicates, as would be expected, that the intervals for the mean of lines selected alike are smaller than those for the means of individual lines.

## DISCUSSION

Because of the small effective number of parents used to perpetuate each line and because of the culturing methods employed, it was expected that the variability of response to selection would be large. The results of the preceding analysis confirm this expectation; a definite, but highly variable, response to selection was observed.

It should be emphasized that the magnitude of the variation observed in this experiment is not necessarily to be expected in other selection studies. Each line

TABLE 7

*Confidence intervals for the mean of lines of Drosophila selected alike*

| Generation of selection | Selection group | | | | |
|---|---|---|---|---|---|
| | U10% | D10% | U25% | D25% | 100% |
| 0 | ..... | ..... | ..... | ..... | 815–880 |
| 1 | 690–776 | 694–755 | 690–797 | 659–753 | 708–794 |
| 2 | 650–790 | 654–732 | 671–799 | 688–743 | 697–785 |
| 3 | 673–834 | 653–728 | 712–817 | 603–749 | 680–747 |
| 4 | 644–779 | 629–700 | 683–817 | 632–788 | 692–788 |
| 5 | 691–788 | 622–743 | 642–746 | 665–786 | 682–785 |
| 6 | 680–819 | 592–714 | 684–777 | 573–715 | 682–817 |
| 7 | 635–816 | 633–748 | 624–784 | 602–703 | 674–766 |
| 8 | 643–810 | 509–734 | 657–757 | 610–759 | 640–778 |
| 9 | 685–796 | 460–840 | 681–860 | 466–802 | 662–768 |
| 10 | 735–919 | 568–710 | 562–815 | 474–798 | 629–763 |

was reared in only one vial per generation and this could add to the interline component if intervial differences are important. Also, a decrease in the contemporaneity among lines in the last few generations may have contributed to the large interline component found in these generations. Thus, because of the small effective number of parents used to perpetuate the lines and because of the culturing methods employed, it is possible that the interline variance components estimated in this study may approach an upper limit for studies in which all lines of a particular generation are reared contemporaneously.

As mentioned earlier, the expected response to selection may be estimated if the heritability of the characteristic and the selection differential are known. This expectation, however, does not adequately take into account the random variation involved. It would seem that a more realistic estimate of the expected genetic gain could be obtained by attaching the respective intervals presented in equations 4–6 to the product of the heritability and the selection differential. However, such predictions are valid only when intergeneration differences in variance components, level of environment and effect of inbreeding are accounted for. These conditions were not accounted for in the present study. Such predictions could be useful, however, in selection studies in which these conditions are accounted for and in which it is feasible to calculate the interline and intraline components of variance.

It is felt that estimates of the interline and intraline variance components using various effective numbers of parents would be useful in determining the optimum number of replicates to include in a selection experiment and the optimum effective number of parents to perpetuate each line. Such a study is currently underway in our laboratory.

In general, it is concluded that the response to selection in this experiment was highly variable; thus, under the conditions of the experiment, random variation is indeed an important source of variation. However, it should be remembered that variability in the response to selection should be at a maximum in this experiment where the lines were perpetuated by full-sib matings and an effective population size of two. It would seem that selection studies involving various effective numbers of parents per line, various characteristics and various organisms are necessary to determine the generality of the variability of response to selection.

## SUMMARY

The results of this investigation may be summarized as follows: (1) Selection for body weight at two intensities in each of two directions was accompanied by a definite, but variable, response when the lines were perpetuated by only two parents which were full-sib mated. (2) Regressions of response to selection on cumulative selection differentials indicated that the heritability of body weight of *Drosophila affinis* is approximately 0.06; this estimate agrees quite closely with those determined from pooled parent-offspring regressions. (3) Confidence intervals using estimates of the interline and intraline components of variance

were employed to describe the variability of response to selection and they may be attached to the expected response to selection. (4) Random or chance variation was important under the conditions of the present study. (5) Experimentation is necessary to determine the generality of the variability of response to selection in different organisms, different characteristics and with different effective numbers of parents.

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# RELEASE OF GENETIC VARIABILITY THROUGH RECOMBINATION. VII. SECOND AND THIRD CHROMOSOMES OF DROSOPHILA MELANOGASTER

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THE earliest experiments reported in this series (SPASSKY, SPASSKY, LEVENE and DOBZHANSKY 1958; SPIESS 1959; and DOBZHANSKY, LEVENE, SPASSKY and SPASSKY 1959) leave no doubt that a substantial portion of the genetic variation controlling viability in wild populations of Drosophila species arises from intrachromosomal recombination, even when the recombined chromosomes are selected for their normal viability effects. In all three species tested average viabilities were lower following recombination, and the nonadditive component of variance was consistently significant, facts which indicate considerable epistatic interaction between linked loci controlling viability.

Nevertheless while these effects on average viability and variance must be a consequence of the release of potential variability from heterozygous linked complexes, no one has yet succeeded in measuring relative amounts of heterozygosity nor the magnitude of the linked complexes involved. There is no question that several isoallelic differences must exist between these chromosomes which control apparently equal effects and consequently that the population from which they were extracted is highly heterozygous and likely to be "balanced" (DOBZHANSKY 1955). However the magnitude of change after recombination depends on (1) the number of loci recombined, (2) ability of those loci to recombine, (3) additive effects of those loci, and (4) their epistatic interaction effects both intrachromosomal and interchromosomal. In the series of experiments referred to above, for example, no measure was made of the component of variance contributed by the segregation of unrecombined chromosomes, or interchromosomal interaction (as pointed out to the senior author by F. W. ROBERTSON, personal communication). By measuring viabilities of progeny from $F_1$ males in which no crossing over takes place, a control may be provided to refine the recombination data still further. The experiments reported here employ this additional control. The hope of ascertaining the number of loci recombined and their ability to recombine seems more remote, although the approach of BREESE and MATHER (1957, 1960) to this problem gives some assurance of ultimate solution.

In *D. pseudoobscura* the range of variation produced following recombination

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was great enough to include extremes of viability such as lethality ("synthetic lethals": DOBZHANSKY 1946; SPASSKY *et al.* 1958). Some lethals so produced were tested for linkage position with markers on chromosome II and shown to be "synthetic" rather than positional (DOBZHANSKY and SPASSKY 1960).

WALLACE *et al.* (1953) reported statistical evidence for synthetic lethals on chromosome II of *D. melanogaster* as did MISRO (1949). WIGAN (1949) demonstrated the synthetic nature of some of MISRO's lethals but his paper could not be critically evaluated since it was only an abstract and unfortunately did not anticipate the problems to follow. Considerable doubt was cast on the existence of synthetic lethals in *D. melanogaster* by HILDRETH (1955, 1956) when he was unable to demonstrate them on the X and third chromosomes of that species. The X chromosome might be expected to give negative results except for lethals limited to expression in females. HILDRETH's negative results for chromosome III are more difficult to explain. Unfortunately the issue was made no clearer by the experiments of GANTNER (1958) in which single second chromosomes were recombined with a marker chromosome, a procedure which would have small likelihood of releasing a lethal as an extreme variant of recombination between two naturally occurring genomes.

SPIESS (1958) suggested that the WALLACE-MISRO-WIGAN results for chromosome II *vs.* HILDRETH's for the X and third chromosomes might be resolved if the second chromosome was in fact different in its ability to release extreme recombinants. BREESE and MATHER (1960) drew attention to the highly significant interactions for viability on chromosome III which they observed and they quite correctly pointed out that their earlier results with the morphological character (chaeta number) which showed very low interaction on chromosome III was a feature of the character, not of the chromosome.

It appeared worthwhile to reinvestigate the effects of recombination on viability in *D. melanogaster* using the experimental design of the first papers in this series. "Normal viability" chromosomes were extracted from a random mating, large laboratory population which had been maintained for more than 150 generations (see below). Viability effects on both chromosomes II and III were selected simultaneously. Parallel experiments were run using progeny from $F_1$ males or $F_1$ females as the case may be for determining the effects of chromosomal assortment as well as intrachromosomal recombination. Greater accuracy of variance increment produced by the latter was thereby attained. Also by testing both chromosomes simultaneously, any differences in background genotype were limited to about one fifth of the total genome (X and fourth chromosomes). Production of synthetic lethals could be compared in the two parallel experiments (in the manner of MISRO's comparison). In addition it was planned that any lethals which might arise could easily be tested for linkage position. It was with the hope of helping to clarify the conflicting evidence and at the same time refining the methods of measuring the effects due to recombination by keeping a control on interchromosomal interactions and on polygenic mutation that these experiments were undertaken.

## MATERIALS AND METHODS

Flies were taken from a population derived from WALLACE's (1950) control population 3, which had in turn been initiated from 16 strains of *Drosophila melanogaster* (Oregon R) homozygous for chromosome II. WALLACE used second chromosomes of near normal viability for all strains, that is, they were devoid of lethals and semilethals. Population 3 was introduced into a population cage on June 25, 1949 by combining a heterogeneous mixture of all 16 strains and was maintained until 1958. Twenty egg samples were kindly contributed by WALLACE to SPIESS's laboratory during the summer of 1958. All progeny from these eggs were introduced into a population cage at 25°C and have been maintained to the present date by adding two new food creamers three times per week. According to the observed generation time under these population cage conditions it requires an average of 21 days per generation. The population was maintained in this laboratory for three generations before the start of the experiment, and WALLACE had maintained it for 156 generations, making a total of 159 generations from the time population 3 was started.

The balancer stock was obtained through the courtesy of WALLACE and KING at Cold Spring Harbor, New York, in 1958: Chromosome II, *In(2LR)Cy, al² Cy lt¹ L⁴ sp²/Pm*; and chromosome III, *In(3LR)Ubx¹³⁰, Ubx¹³⁰ e⁸/l(3)tr Sb*. This stock will be referred to as *Cy L/Pm, Ubx/Sb*.

In order to obtain wild-type flies with second and third chromosomes of normal viability, a series of four egg samples were taken from the population cage. The first two samples were taken two days apart, and two months later (about three generations) the next two were taken eight days apart. Egg samples were allowed to develop in well yeasted half pint food bottles. Twenty-five males from each sample bottle were mated singly to *Cy L/Pm, Ubx/Sb* females as indicated below:

| | II | III | | II | III |
|---|---|---|---|---|---|
| $P_1$ | +a/+b | +x/+y ♂ | × | *CyL/Pm* | *Ubx/Sb*♀♀ |

$F_1$ *CyL*/+a (or +b), *Ubx*/+x (or +y) ♂ × *CyL/Pm, Ubx/Sb*♀♀

$F_2$ *CyL*/+a, *Ubx*/+x ♂♂ × *CyL*/+a, *Ubx*/+x♀♀ (sib mating)

| $F_3$ Classes | Expected Number |
|---|---|
| *CyL*/+a, *Ubx*/+x | 45 |
| *CyL*/+a, +x/+x | 22 |
| +a/+a, *Ubx*/+x | 22 |
| +a/+a, +x/+x | 11 |
| | 100 Total |

where +a (or +b) represents any second chromosome and +x (or +y) represents any third chromosome from the population cage. (Letters representing any chromosome are replaced with strain numbers in the plan of experiments of Figure 1.) Single $F_1$ males were crossed with the balancer females to isolate the "wild" chromosomes. $F_2$ brothers and sisters were mated, their non-Curly-Lobe, non-*Ubx* chromosomes being identical. Four classes of $F_3$ flies were expected as

shown above, of which exactly 100 were counted and classified. Since the markers *Cy* and *Ubx* are homozygous lethals, each marker is expected to appear in a 2:1 ratio. By testing both chromosomes simultaneously the joint ratio of 4/9:2/9:2/9:1/9 was expected. Viability values were designated lethal if no non-*Cy* or non-*Ubx* flies appeared in a culture and semilethal if less than 15 but more than zero of the respective nonmarker flies appeared for either chromosome.

From the original 100 males tested, ten strains were selected with near normal viability values (nearest 33 percent nonmarkers for either chromosome), each strain from one male. Strains were maintained in the balanced condition (*Cy L*/+, *Ubx*/+) both by single pair matings and by mass matings of *Cy L*, *Ubx* flies only. Single pair matings served to check viability values from generation to generation and to minimize variation during the course of the experiment arising from mutation. Mass matings were kept for insurance purposes in the event of a loss or mutational change in the single pair matings. In addition to this generation check, viability values for each strain were determined in more detail at the beginning, about midway, and at the end of the main experiments by subculturing each strain six times. A measure of environmental effects and mutation within strains could thereby be made. Control viability values for strains were averaged from these subculture tests.

Only one lethal arose by mutation in the strains during the course of the experiment: in strain 4 on chromosome III. This chromosome was reisolated from mass culture 4 and that part of the experiment involving strain 4 was repeated. However, after reisolation of strain 4, the third chromosome showed below normal viability values and was excluded in certain analyses.

All strains were checked for chromosomal aberrations by examination of salivary gland chromosomes. Wild-type males of one strain were mated with females of another strain, and salivary preparations in acetic orcein were made on $F_1$ larvae. All second and third chromosomes were found free of visible aberrations.

In order to determine viability differences after crossing over and recombination between second and third chromosomes, the ten strains were crossed in every possible combination, making a total of 45 crosses. The plan of the experiments is given in Figure 1. Strains 5 and 4 are chosen arbitrarily for the mating scheme and represent a cross between any two strains (1 through 10). By testing chromosomes II and III simultaneously, chromosomal background effects were minimized with about four fifths of the total chromosomal complement being marked.

Under the $F_1$ ♀ ♀ plan (see Figure 1) crossing over can occur between wild chromosomes of the ten strains. Recombinant chromosomes were then collected and counted as shown. Under the $F_1$ ♂ ♂ plan in which crossing over did not occur, any effect of isoallelic segregation, assortment to new genetic backgrounds, or mutation on viability could be observed. Chromosomes derived from the $F_1$ ♀ ♀ plan will be termed "recombinant" and those from the $F_1$ ♂ ♂ plan "non-recombinant" in this paper.

To measure variance within each cross a sample of ten $F_2$ males was tested by

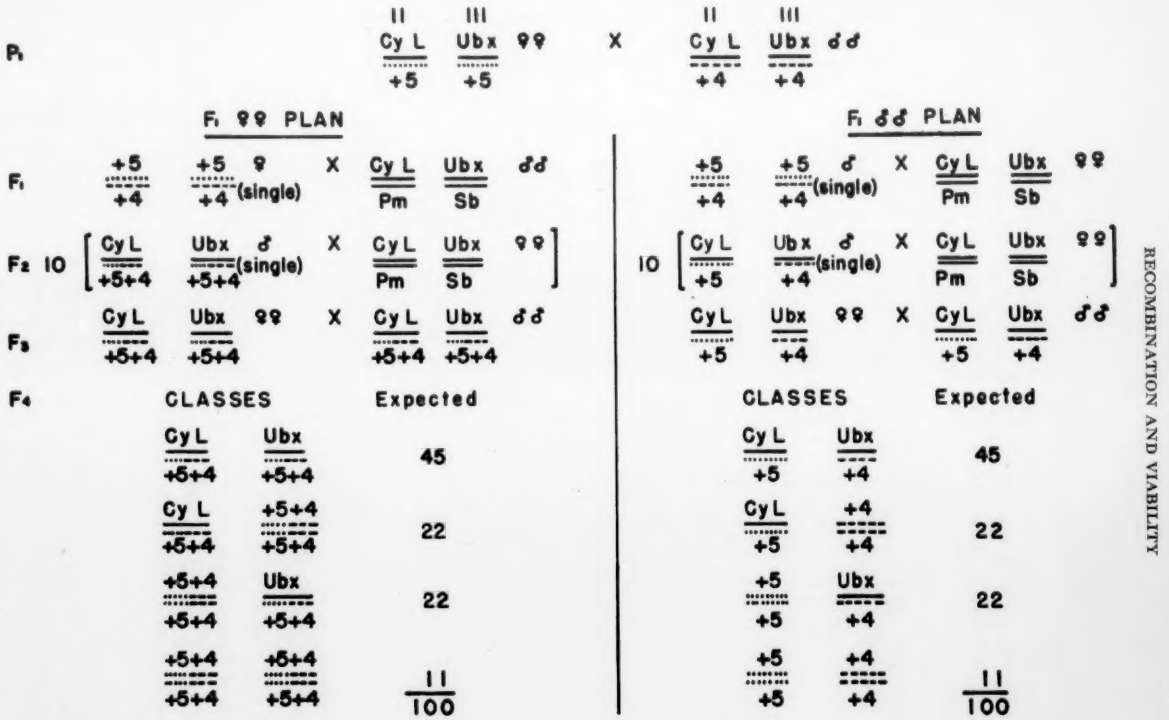
P1
II III
Cy L Ubx ♀♀ X II III
+5 +5 Cy L Ubx ♂♂
+4 +4
F1 ♀♀ PLAN
F1 +5/+4 +5/+4 ♀ (single) X Cy L/Pm Ubx/Sb ♂♂
F2 10 [Cy L/+5+4 Ubx/+5+4 ♂ (single) X Cy L/Pm Ubx/Sb ♀♀]
F3 Cy L/+5+4 Ubx/+5+4 ♀♀ X Cy L/+5+4 Ubx/+5+4 ♂♂
F4 CLASSES Expected
Cy L/+5+4 Ubx/+5+4 45
Cy L/+5+4 +5+4/+5+4 22
+5+4/+5+4 Ubx/+5+4 22
+5+4/+5+4 +5+4/+5+4 11
100
F1 ♂♂ PLAN
+5/+4 +5/+4 ♂ (single) X Cy L/Pm Ubx/Sb ♀♀
10 [Cy L/+5 Ubx/+4 ♂ (single) X Cy L/Pm Ubx/Sb ♀♀]
Cy L/+5 Ubx/+4 ♀♀ X Cy L/+5 Ubx/+4 ♂♂
CLASSES Expected
Cy L/+5 Ubx/+4 45
Cy L/+5 +4/+4 22
+5/+5 Ubx/+4 22
+5/+5 +4/+4 11
100

FIGURE 1.—Plan of matings for testing the viability of wild second and third chromosomes after crossing over ($F_1$ ♀ ♀ plan) and no crossing over ($F_1$ ♂ ♂ plan) for ten strains of normal viability. Recombinant chromosomes are represented by +5 +4, or crossover chromosomes between strains 5 and 4; +5 or +4 are nonrecombinant chromosomes from strains 5 or 4.

collecting homozygous wild chromosomes in the $F_4$ generation. The first 100 flies counted in $F_4$ measured viability without regard for order of emergence. Consequently a total of 900 chromosomes were tested (450 from crossovers, 450 from noncrossovers) for viability values by counting 90,000 flies. Analyses of variance were carried out using LEVENE's (1959) design on recombinant and non-recombinant chromosomes separately. Comparisons between recombinant, non-recombinant, and control chromosomes were made with method of "t".

All cultures were maintained in half pint milk bottles on SPASSKY's (1943) cream-of-wheat molasses medium with bakers' yeast added. All experiments were carried at 25° ± 1°C.

## RESULTS

*Viability values for the laboratory population:* The range in viability for 100 second and third chromosomes from the population which was the source of variability used in this experiment is given in Figure 2. The abscissa indicates the percentage of non-*Cy L* or non-*Ubx* flies for the second and third chromosome viabilities respectively; the ordinate gives the number of chromosomes for each viability value. Crosshatched columns represent second chromosomes and plain columns, third chromosomes. Both chromosomes displayed a wide diversity of viabilities, and the shape of the curve closely resembles that typical of samples from many wild populations. It should be noted again that this population has been initiated with flies isogenic for chromosome II and maintained under constant laboratory conditions for more than 150 generations. Lethal-bearing chromosomes were more prevalent among third chromosomes than second (27 lethal third chromosomes compared with 16 lethal second chromosomes). Most of the

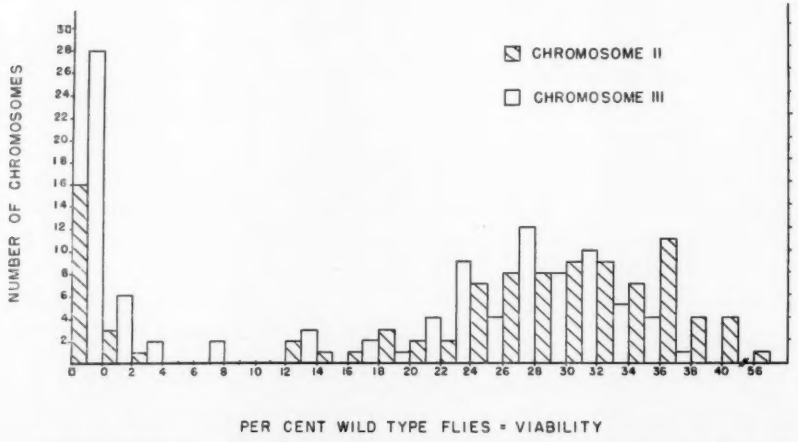


FIGURE 2.—Relative viability of second and third chromosomes extracted from the laboratory population.

chromosomes, excluding those with lethals and semilethals with viability values below 12, were distributed around a value just below "normal" viability (33 percent wild type): for chromosome II these averaged 32.3 ± 0.84, while third chromosomes have lower viability with an average of 28.2 ± 0.68. The difference between these chromosomes is significant ($P < .01$, $t = 3.63$, d.f. = 141, with 79 for II and 62 for III). Ten strains were chosen with near normal viability for both chromosomes II and III simultaneously to be used in the following recombination experiment.

*Viability values for chromosome II after crossing:* Table 1 is constructed to show the average viability values for homozygous second chromosomes following strain hybridization from 45 crosses without intrachromosomal (crossing over) recombination, 45 crosses with intrachromosomal recombination, and ten controls. The strain numbers are located at the top margin and along the left side of the table.

Control average viability values run diagonally across the table and have been blocked in. The mean viability value for second chromosome controls was 32.3 ± 0.73, which is the same as the average viability value for population cage second chromosomes (32.3 ± 0.84) with a smaller standard error.

The average viability values for homozygous second chromosomes from crosses between the ten strains in which crossing over (intrachromosomal recombination) occurred (see Figure 1, $F_1$ ♀ ♀ Plan) are found in the upper right half of the table. These homozygous chromosomes which shall be designated "recombinant" had a grand mean viability value of 33.2 ± 0.71.

The average viability values for homozygous second chromosomes for 45 crosses in which no crossing over (intrachromosomal recombination) occurred

TABLE 1

*Average viability for chromosome II with and without crossing over between ten strains*

| | Recombinant homozygote viabilities | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Non-recombinant homozygote viabilities — Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\bar{X}$ |
| 1 | 35.6 | 28.7 | 30.4 | 33.8 | 30.1 | 33.0 | 34.0 | 33.5 | 33.0 | 39.3 | 32.3 |
| 2 | 31.5 | 31.8 | 36.6 | 33/7 | 32.0 | 29.9 | 30.6 | 28.5 | 33.8 | 31.7 | 31.7 |
| 3 | 33.2 | 27.2 | 33.8 | 42.0 | 33.1 | 38.8 | 25.7 | 27.6 | 40.4 | 31.6 | 34.0 |
| 4 | 31.5 | 32.6 | 21.9 | 29.7 | 40.4 | 28.2 | 32.1 | 30.3 | 31.9 | 30.5 | 33.7 |
| 5 | 28.0 | 28.1 | 31.0 | 31.4 | 32.8 | 28.3 | 31.8 | 32.4 | 38.0 | 43.7 | 33.8 |
| 6 | 33.1 | 29.5 | 33.8 | 30.0 | 31.2 | 29.8 | 30.9 | 30.0 | 31.5 | 41.5 | 32.5 |
| 7 | 26.5 | 29.3 | 33.1 | 32.6 | 31.5 | 32.9 | 30.1 | 32.1 | 35.0 | 33.7 | 31.8 |
| 8 | 33.5 | 28.9 | 27.3 | 28.0 | 30.0 | 31.3 | 31.1 | 29.3 | 34.2 | 26.7 | 30.6 |
| 9 | 31.9 | 29.6 | 29.7 | 33.4 | 31.3 | 33.1 | 35.3 | 27.5 | 34.9 | 34.7 | 35.8 |
| 10 | 29.7 | 28.8 | 25.8 | 33.7 | 29.5 | 29.7 | 30.4 | 31.5 | 32.6 | 34.8 | 35.9 |
| | | | | | | | | | | | $\hat{m}$ = 33.2 |
| $\bar{X}$ | 30.98 | 29.5 | 29.2 | 30.6 | 30.2 | 31.6 | 31.4 | 29.9 | 31.6 | 30.2 | $\hat{m}$ = 32.3 |
| | | | | | | | | | $\hat{m}$ = 30.5 | | |

(see Figure 1, $F_1$ ♂ ♂ Plan) are found in the lower left half of Table 1. These homozygous chromosomes which shall be designated "non-recombinant" had a mean viability value of 30.5 ± 0.37.

In the mean viability value ($\overline{X}$) column on the right margin of Table 1 it can be seen that the average effect of recombining each chromosome with all the others was an increase in the viability above its control value, except for the average effect of strain 1 which was decreased from 35.6 to 32.3 and the effect of strain 2 which showed almost no effect (31.8 to 31.7). Homozygous recombined second chromosomes increased by an average of 0.95 over control. This increase has a probability of 16 percent, $t = 1.57$ with nine degrees of freedom. If the average effect of strain 1 is omitted, however, the recombined remainder shows an increase in viability of 1.42 over the original chromosomes, and that increase is highly significant ($t = 3.36$ with 8 degrees of freedom, $P = 0.01$). Omission of strain 1 can be justified in the following way: its original viability had been selected far higher than any other chromosomal strain. It could be stated that for all "normal" chromosomes with original viabilities of 34.9 or lower the tendency was to increase in viability following intrachromosomal recombination.

Mean viability values ($\overline{X}$) for non-recombinant chromosomes are shown along the lower margin in Table 1. There was an average decrease in viability of 1.74 for unrecombined homozygous chromosomes derived from $F_1$. This decrease has a probability of 6.5 percent, $t = 2.09$ with nine degrees of freedom and is not significant. Out of ten mean values, four increase and six decrease, so that there is no reason to believe that segregation of normal second chromosomes in $F_1$ males consistently affected the total viability average.

The average effect of crossing over (or intrachromosomal recombination) when compared with the effect of entire chromosome assortment (no crossing over) was a significant average increase of 2.79 in viability after crossing over ($P < 0.01$, $t = 4.887$ with nine degrees of freedom). All mean viability values for recombinants were higher than their corresponding non-recombinant values. This is probably a better comparison than recombinants *vs.* controls, because the effects of segregation of entire chromosomes would not be expressed in homozygous controls as they must be in the $F_1$ males plan. Since both segregation and crossing over occur in $F_1$ females, the $F_1$ males data have been used as a control for recombinant chromosomes estimating the effects of crossing over on viability. The original chromosomes can be used as controls for non-recombinant chromosomes, estimating the difference between isogenic variability (zero genetic variation) and variability arising from segregation of heterogeneous "normal" chromosomes.

*Viability values for chromosome III after crossing:* Table 2 is constructed identically with Table 1 and it includes average viability values for homozygous chromosomes. Control average viability values running diagonally across the table have a mean viability value of 30.6 ± 0.89 for chromosome III. This value may be compared with 28.2 ± 0.68 for population cage third chromosomes tested.

TABLE 2

*Average viability for chromosome III with and without crossing over between ten strains*

| | | Recombinant homozygote viabilities | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\bar{X}$ |
| Non-recombinant homozygote viabilities | 1 | 32.2 | 24.8 | 28.7 | 24.0 | 33.7 | 31.0 | 28.0 | 33.0 | 26.9 | 31.3 | 28.7 |
| | 2 | 28.0 | 27.3 | 31.4 | 25.8 | 25.3 | 23.7 | 32.5 | 29.5 | 25.5 | 28.6 | 27.5 |
| | 3 | 28.4 | 26.9 | 29.7 | 18.1 | 34.5 | 30.0 | 28.3 | 29.0 | 33.0 | 30.7 | 29.3 |
| | 4 | 24.5 | 25.5 | 31.8 | 23.6 | 17.3 | 26.9 | 24.9 | 24.7 | 24.4 | 22.1 | 23.1 |
| | 5 | 31.2 | 23.4 | 35.7 | 25.4 | 31.8 | 30.3 | 27.4 | 26.2 | 37.5 | 33.2 | 29.5 |
| | 6 | 28.5 | 24.2 | 29.0 | 30.3 | 26.8 | 32.2 | 29.4 | 29.9 | 24.2 | 27.2 | 28.1 |
| | 7 | 28.7 | 33.5 | 32.8 | 28.1 | 31.5 | 29.2 | 32.2 | 32.4 | 31.2 | 31.9 | 29.2 |
| | 8 | 27.5 | 27.8 | 29.5 | 22.5 | 31.4 | 26.8 | 33.5 | 31.8 | 23.8 | 32.2 | 28.9 |
| | 9 | 27.1 | 20.0 | 30.4 | 23.7 | 22.7 | 30.2 | 29.6 | 28.0 | 31.9 | 30.1 | 28.5 |
| | 10 | 29.2 | 29.6 | 26.2 | 23.4 | 30.0 | 26.4 | 28.3 | 24.9 | 27.8 | 33.1 | 29.7 |
| | | | | | | | | | | | | $\hat{m} = 28.3$ |
| | $\bar{X}$ | 28.12 | 26.5 | 30.1 | 26.1 | 28.7 | 27.9 | 30.6 | 27.9 | 26.6 | 27.3 | $\hat{m} = 30.6$ |
| | | | | | | | | | | | $\hat{m} = 27.9$ | |

The chromosomes chosen for recombination studies were higher in viability therefore by an average value of 2.4.

Recombinant homozygotes in the upper right half of Table 2 have a grand mean viability value of 28.3 ± 0.62. The lower left half of the table shows non-recombinant homozygotes with a grand mean viability value of 27.9 ± 0.48. Both recombinant and non-recombinant chromosomes approach average viabilities of the population cage average for third chromosomes.

A comparison of mean viability values of recombinant third chromosomes and controls reveals a significant decrease in viability of 2.3 after recombination ($P < .01$, $t = 4.75$ with 9 degrees of freedom). All recombinant means show a decrease when compared with controls, except strain 2 which had almost no effect (27.5 to 27.3). This decrease in viability for third recombinant chromosomes contrasts with the second chromosome results which tended to increase following recombination.

Non-recombinant third chromosomes also show a significant decrease of 2.6 in mean viability values below controls ($P < .01$, $t = 3.88$ with 9 degrees of freedom). All chromosome means are below controls except strain 3 (29.7 to 30.1) and strain 4 (23.6 to 26.1) which show increases in viability. Strain 4 had a sub-vital chromosome III however (see below), and can be considered exceptional.

A comparison between recombinant and non-recombinant third chromosome mean viability values indicates no significant difference ($P = 0.65$, $t = 0.54$ with 9 degrees of freedom). There was a difference of only 0.4 between their grand means. Therefore the lowering of viability observed in recombinant chromo-

somes when compared with controls cannot be ascribed to intrachromosomal recombination only but follows assortment of whole chromosomes to new genetic backgrounds.

*Viability values for chromosomes II and III together:* It can be seen in Figure 1 that the number of double homozygous wild-type flies per 100 counted was expected to be 11. The observed values for these numbers, based on 11 as being normal, is an expression of interaction between chromosomes II and III.

Table 3 is constructed identically with Tables 1 and 2 but it includes average viability values for homozygous second and third chromosomes together (percent of wild-type flies). The control viability values have a grand mean of $10.33 \pm 0.39$, omitting strain 4. When strain 4 was included the grand mean was $9.63 \pm 0.75$ for controls (see below strain 4). Therefore, with an average effect to lower viability by 0.7, strain 4 has been omitted from the calculations for t values when making comparisons between recombinants, non-recombinants and controls for double homozygotes. The grand mean viability value for recombination data (including 4) was $9.14 \pm 0.34$. Non-recombination data (including strain 4) had a grand mean of $8.08 \pm 0.21$.

The average viability values for double homozygous chromosomes both with recombination (9.28 omitting strain 4) and without recombination (8.14 omitting strain 4) show a significant decrease in viability below controls ($P < .01$, $t = 4.78$ with 8 degrees of freedom for recombinants). When recombinants are compared with non-recombinants, there is a significant increase in the viability of recombinants ($P < .01$, $t = 4.07$ with 8 degrees of freedom). Therefore, reassortment of whole chromosomes has the greater lowering effect, and intra-

TABLE 3

*Average viability for chromosome II and III together with and without crossing over between ten strains*

| Non-recombinant homozygote viabilities: Strain | Recombinant homozygous viabilities: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\bar{X}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11.0 | 7.5 | 9.2 | 7.6 | 8.6 | 8.9 | 8.9 | 10.8 | 9.9 | 13.0 | 9.4 |
| 2 | 8.9 | 8.8 | 10.6 | 8.6 | 7.9 | 6.4 | 10.1 | 8.5 | 8.2 | 9.0 | 8.2 |
| 3 | 9.4 | 6.0 | 10.8 | 9.4 | 10.4 | 11.9 | 7.2 | 7.8 | 14.6 | 9.6 | 10.1 |
| 4 | 7.6 | 8.1 | 7.1 | 3.3 | 8.2 | 7.9 | 7.8 | 7.7 | 7.5 | 6.8 | 7.9 |
| 5 | 6.8 | 5.6 | 11.7 | 7.7 | 10.7 | 7.5 | 7.9 | 6.8 | 12.2 | 14.9 | 9.4 |
| 6 | 10.0 | 7.3 | 10.2 | 8.3 | 8.2 | 8.9 | 10.0 | 8.3 | 7.0 | 8.6 | 8.5 |
| 7 | 6.9 | 9.2 | 10.5 | 9.5 | 7.9 | 9.5 | 10.6 | 9.5 | 9.9 | 10.2 | 9.1 |
| 8 | 9.1 | 7.2 | 7.4 | 5.8 | 8.0 | 7.1 | 8.8 | 9.4 | 6.3 | 8.3 | 8.2 |
| 9 | 9.3 | 5.8 | 8.5 | 7.7 | 6.2 | 9.0 | 8.6 | 5.8 | 10.0 | 13.4 | 9.9 |
| 10 | 8.1 | 8.0 | 8.0 | 6.1 | 10.1 | 7.5 | 8.2 | 7.6 | 9.2 | 12.8 | 10.4 |
| | | | | | | | | | | | $\hat{m} = 9.14$ |
| $\bar{X}$ | 8.5 | 7.3 | 8.8 | 7.5 | 8.0 | 8.6 | 8.8 | 7.4 | 7.8 | 8.1 | $\hat{m} = 9.63$ |
| | | | | | | | | | | $\hat{m} = 8.08$ | |

chromosomal recombination partially restores viability in the direction of the control average.

*Strain 4 viability value—chromosome III:* As mentioned above a lethal mutation arose on chromosome III in strain 4 during the experiment. After reisolation from the original mass culture this chromosome had an average viability of $23.6 \pm 1.15$, a value which is more than two standard deviations below the mean for all control viability values (mean = 30.6, where $\sigma = 2.81$). Therefore chromosome III in strain 4 was a subvital which had mutated from normal after original isolation of the strain. This subvital's effect is greatest in the double homozygote control viability value (3.3) and has a large statistical effect in viability comparisons and in variance calculation. Therefore strain 4 was omitted from calculations for t values on double homozygote data.

After the production of a lethal in strain 4, viability counts were made on all strains every two generations. No other lethal mutations were produced during the experiment, and viability of all strains remained constant. Homogeneity chi-squares on the controls from generation to generation were nonsignificant for each chromosome. Viability of the dominant markers was also checked by method of t on the difference between the *Cy L* and *Ubx* classes. None of the strains indicated any significant difference between the viabilities of these markers except strain 4, which gave a highly significant excess of *Ubx*.

*Variance in crosses involving chromosomes II, III, and both:* Tables 4, 5, and 6 show the variance for all crosses as well as controls. The variance *(V)* given for each cross equals the crude variance *(U)* minus the binomial sampling variance *(B)* for the ten homozygotes tested in that cross (or for controls, the 12

TABLE 4

*Average within cross variances (V) for chromosome II with and without crossing over between ten strains*

| | | Recombinant homozygote *V* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Non-recombinant homozygote *V* | Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\bar{Y}_{i.}$ |
| | 1 | -14.68 | 8.20 | 0.39 | 5.84 | 44.97 | 120.33 | 24.22 | -3.67 | 6.55 | -4.30 | 22.50 |
| | 2 | 9.75 | 0.31 | 117.46 | 43.66 | 13.57 | 5.59 | 18.09 | -11.05 | 8.73 | -2.54 | 22.41 |
| | 3 | -3.29 | -7.80 | -2.10 | 20.75 | 17.97 | 21.80 | 36.91 | -3.54 | 59.70 | 113.27 | 42.74 |
| | 4 | 2.42 | 3.47 | 32.79 | -7.50 | 47.25 | -11.14 | 26.32 | 31.77 | -2.28 | 40.36 | 22.50 |
| | 5 | 52.51 | -1.20 | 12.54 | -8.76 | -13.21 | 7.43 | 49.42 | 15.43 | 69.10 | 20.06 | 30.12 |
| | 6 | 27.30 | -11.90 | -4.82 | 8.66 | 17.20 | -9.79 | 2.76 | 3.89 | 12.20 | -15.17 | 16.41 |
| | 7 | -1.48 | 11.72 | -3.81 | 2.70 | 2.86 | 11.59 | -2.73 | -2.57 | -3.98 | 9.88 | 17.89 |
| | 8 | 6.39 | 4.01 | 4.15 | -6.38 | 7.00 | -10.17 | 15.57 | -10.59 | -3.17 | 5.31 | 3.60 |
| | 9 | 4.83 | -6.17 | 0.68 | 0.42 | 1.61 | -3.36 | 1.16 | 16.06 | -3.37 | 106.83 | 28.19 |
| | 10 | -12.88 | -2.07 | 10.04 | 51.66 | -7.90 | 40.23 | -1.83 | 0.86 | 44.47 | -3.17 | 30.41 |
| | | | | | | | | | | | | $\hat{m} = 23.68$ |
| | $\bar{Y}_{.j}$ | 9.83 | -0.02 | 4.49 | 9.66 | 8.43 | 8.30 | 4.27 | 4.16 | 6.63 | 13.62 | $\hat{m} = -6.72$ |
| | | | | | | | | | | | $\hat{m} = 6.94$ | |

TABLE 5

*Average within cross variances (V) for chromosome III with and without crossing over between ten strains*

| | Recombinant homozygote *V* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Non-recombinant homozygote *V* / Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\bar{Y}_{ij}$ |
| 1 | 25.71 | -1.98 | -1.13 | -4.91 | 24.77 | 35.72 | 128.34 | -6.78 | 18.01 | 21.16 | 23.67 |
| 2 | 25.84 | -12.51 | 37.13 | 20.19 | 15.99 | 1.47 | 4.95 | 13.21 | 0.77 | 11.02 | 11.42 |
| 3 | -3.22 | 2.67 | -6.47 | 59.29 | -1.26 | 5.66 | -2.29 | 7.85 | 52.77 | 82.06 | 26.67 |
| 4 | -1.17 | 0.67 | -2.14 | 1.20 | 92.72 | 2.23 | 27.86 | 35.85 | 49.88 | 120.22 | 44.81 |
| 5 | 20.98 | 2.75 | 13.62 | 7.49 | -5.47 | 20.21 | -1.67 | 31.99 | 123.00 | 58.71 | 40.49 |
| 6 | 40.29 | -4.79 | 3.12 | -2.90 | 16.82 | -10.41 | 34.13 | 0.71 | 19.21 | 27.09 | 16.27 |
| 7 | 11.54 | 60.83 | 17.07 | 60.13 | 14.20 | 21.55 | -7.22 | 1.88 | 12.32 | 25.72 | 25.69 |
| 8 | 12.28 | 35.93 | -2.42 | 5.00 | 7.16 | 14.60 | -12.95 | 6.15 | 8.97 | 23.06 | 12.97 |
| 9 | 16.13 | 19.73 | 16.62 | 31.39 | 6.45 | -6.64 | 27.60 | 38.95 | 2.42 | 11.07 | 32.89 |
| 10 | -4.67 | 7.83 | 23.24 | 56.52 | 9.59 | 47.23 | 3.26 | 12.08 | -5.35 | 0.16 | 42.23 |
| | | | | | | | | | | | $\hat{m} = 27.71$ |
| $\bar{Y}_{ij}$ | 13.11 | 16.83 | 7.62 | 17.22 | 11.01 | 14.36 | 22.58 | 12.29 | 16.09 | 16.64 | $\hat{m} = -0.64$ |
| | | | | | | | | | | $\hat{m} = 14.77$ | |

TABLE 6

*Average within cross variances (V) for chromosomes II and III together with and without crossing over between ten strains*

| | Recombinant homozygote *V* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Non-recombinant homozygote *V* / Strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | $\bar{Y}_{ij}$ |
| 1 | 6.4 | 4.7 | 4.7 | - 2.3 | 2.7 | 0.3 | 18.3 | - 3.8 | -5.5 | 1.1 | 2.2 |
| 2 | 6.9 | -2.7 | 8.9 | 5.3 | 1.3 | 0.9 | -4.1 | 4.2 | -4.2 | -4.2 | 1.4 |
| 3 | -0.9 | 5.1 | 1.1 | 8.0 | -6.6 | 6.5 | 1.1 | -1.7 | 41.3 | 9.7 | 7.9 |
| 4 | -4.1 | 1.6 | 1.1 | 0.7 | 28.1 | -1.2 | 3.7 | 22.7 | 4.4 | 5.2 | 8.2 |
| 5 | 6.4 | -2.3 | -7.5 | 2.5 | 2.7 | 1.7 | 1.5 | 4.1 | 18.9 | 18.7 | 7.8 |
| 6 | 4.3 | -3.2 | 5.0 | 3.4 | 4.7 | -0.2 | 4.0 | 1.7 | -0.3 | 4.7 | 2.0 |
| 7 | -0.3 | 21.6 | -0.1 | 2.7 | -0.1 | 1.6 | 1.1 | -2.8 | 1.4 | 19.0 | 4.7 |
| 8 | -1.9 | 14.3 | 2.9 | 3.7 | -0.3 | 3.4 | 2.4 | 0.5 | -0.4 | 10.2 | 3.8 |
| 9 | 2.3 | -1.7 | 2.6 | 4.9 | 2.4 | -2.0 | 3.3 | 3.8 | 5.4 | 18.6 | 8.2 |
| 10 | -2.4 | -0.1 | 1.1 | 10.9 | -4.1 | 7.5 | 6.9 | 1.0 | 22.4 | 11.2 | 9.2 |
| | | | | | | | | | | | $\hat{m} = 5.54$ |
| $\bar{Y}_{ij}$ | 1.1 | 4.7 | 1.0 | 2.9 | 0.2 | 2.7 | 4.2 | 3.3 | 4.2 | 6.0 | $\hat{m} = 2.62$ |
| | | | | | | | | | | $\hat{m} = 3.03$ | |

homozygotes tested for each strain, six midway samples and six at termination of the experiment). Variances greater than zero are "real," or they imply positive genetic diversity measurably greater than sampling error (see LEVENE 1959, for

details of the statistical design). The tables for variance values are constructed identically with those for viability values. Variance values for controls run diagonally across the tables, recombination variance values appear in the upper right half of the tables, and non-recombination variance values in the lower left half of the tables. The mean variance values ($\overline{Y}_{ij}$) appear in the last right hand column for recombination, in the bottom row for non-recombination, and the grand means ($\hat{m}$) in the right lower corner. Table 4 shows the contribution to variance for chromosome II, Table 5 for chromosome III, and Table 6 for both chromosomes simultaneously.

All three tables show similar results in that those crosses in which recombination had occurred have significantly higher variance values than either controls or crosses without recombination ($P < 0.01$ for all comparisons, except $P = 0.03$ for recombinants *vs.* controls and 0.05 for recombinants *vs.* non-recombinants in Table 6 which involves double homozygous chromosomes). In all comparisons controls show the least contribution to variance, and non-recombinant chromosomes show an intermediate value between controls and recombinant chromosomes. These comparisons indicate that there is more variation engendered by intrachromosomal recombination than by segregation and mutation. However, segregation through the use of $F_1$ males produces significantly high variance in every case. The total real genetic variance produced by intrachromosomal recombination between the ten "normal" chromosomes of each strain was estimated by subtracting the grand mean ($\hat{m}$) variance of non-recombinant chromosomes from the grand mean variance of recombinant chromosomes. These results indicated + 16.74 net recombination variance for second chromosomes, + 12.58 for third chromosomes, and + 2.51 for double homozygous chromosomes.

*Additive and nonadditive effects of viability for chromosomes II and III:* The additive effect of any chromosome is the difference between the grand mean viability and the mean viability of the crosses in which that chromosome was involved. In other words it shows how much viability is affected by simple additive (plus or minus) genes on that chromosome. The additive effect of intrapopulation crosses is 9/8 that difference (WALLACE *et al.* 1953, and papers in this series.) Therefore, $a_i = 9/8\ (Y_i - \hat{m})$ when $a_i$ = additive effect of any chromosome, $Y_i$ the mean viability of that chromosome, and $\hat{m}$ the grand mean.

If viability were determined completely by additive contributions of chromosomes, the mean viability for all crosses could be calculated if the grand mean and additive values of all the chromosomes were known. The difference between these calculated values and what was observed is the nonadditive, or interaction effect, which measures the influence any particular combination of two chromosomes has on viability. It is measured by subtracting the additive effects of both chromosomes in a cross and the grand mean from the average viability of the cross ($g_{ij} = \overline{Y}_{ij} - a_i - a_j - \hat{m}$), where $g_{ij}$ = interaction, or special combining ability of two particular chromosomes, $\overline{Y}_{ij}$ = average viability of ten samples from a cross, $a_i$ or $a_j$ = additive effects of each chromosome, and $\hat{m}$ = the grand mean of all crosses.

TABLE 7

*Analyses of variance for recombinant viabilities*

| Description | Sum of squares | d.f. | Mean sq. | F | P(%) |
|---|---|---|---|---|---|
| | | Second chromosomes | | | |
| Additive | 34.90 | 9 | 3.88 | 0.22 | > 10 |
| Interaction | 622.50 | 35 | 17.79 | 31.2 | <0.1 |
| Within | | 405 | 0.57 | 1.58 | <2.5 |
| Total residual | | 110 | 0.361 | | |
| | | Third chromosomes | | | |
| Additive | 36.79 | 9 | 4.09 | 0.35 | > 10 |
| Interaction | 413.67 | 35 | 11.82 | 19.70 | <0.1 |
| Within | | 405 | 0.60 | 2.29 | <0.1 |
| Total residual | | 110 | 0.262 | | |
| | | Second and third chromosomes | | | |
| Additive | 8.18 | 9 | 0.91 | 0.26 | > 10 |
| Interaction | 120.72 | 35 | 3.45 | 20.29 | <0.1 |
| Within | | 405 | 0.173 | 1.24 | < 10 > 5 |
| Total residual | | 110 | 0.137 | | |

TABLE 8

*Analyses of variance for non-recombinant viabilities*

| Description | Sum of squares | d.f. | Mean sq. | F | P(%) |
|---|---|---|---|---|---|
| | | Second chromosomes | | | |
| Additive | 8.49 | 9 | 0.94 | 0.13 | > 10 |
| Interaction | 246.39 | 35 | 7.04 | 20.11 | <0.1 |
| Within | | 405 | 0.35 | 1.003 | > 10 |
| Total residual | | 110 | 0.349 | | |
| | | Third chromosomes | | | |
| Additive | 25.32 | 9 | 2.81 | 0.35 | > 10 |
| Interaction | 276.70 | 35 | 7.90 | 17.95 | <0.1 |
| Within | | 405 | 0.44 | 1.69 | 1.0 |
| Total residual | | 110 | 0.259 | | |
| | | Second and third chromosomes | | | |
| Additive | 3.77 | 9 | 0.42 | 0.25 | > 10 |
| Interaction | 58.97 | 35 | 1.68 | 12.92 | <0.1 |
| Within | | 405 | 0.13 | 1.032 | > 10 |
| Total residual | | 110 | 0.126 | | |

The results of the analyses of variance for viability of recombinant chromosomes are summarized in Table 7 and non-recombinants in Table 8. The methods of analyses are given by LEVENE (1959). Total variation can be broken down into two parts: First, the genetic components, (1) additive = the average effect of a chromosome in raising or lowering viability of all other combinations which in-

volves that chromosome, (2) nonadditive = interaction of nonallelic loci intrachromosomal or interchromosomal, and (3) variability within crosses from recombining any pair of chromosomes. This latter component should be positive if crossing over releases genetic variability from linked complexes. Secondly, the residual components are those which arise from environmental effects, residual genotype factors, and binomial sampling. The residual variance estimate has 110 degrees of freedom (12 counts were made in each of ten control strains). It is the sum of real residual (control average variance) plus the average sampling error.

Tables 7 and 8 show a consistent nonsignificant additive F value for all recombinant and non-recombinant chromosomes, which indicates that additive effects do not contribute effectively to homozygous variation observed after crossing strains.

F values show consistent significant interaction effects for all chromosomes both recombinant and non-recombinant. All chromosome classes have larger F values in recombinant data than in non-recombinant, which indicates that there is polygenic interaction after formation of new linkages in excess of that produced by segregation and reassortment of whole chromosomes. That is, average viabilities in crosses are not predictable on the basis of additive modifiers. Interaction for chromosome III contrasts with II by producing almost the same F value for third non-recombinants as for recombinants. Therefore, interaction of whole chromosomes seems to have a greater effect in producing variation for chromosome III in this experiment, which may be a real difference between chromosomes II and III in their genetic activity.

The within component of variance (Tables 7 and 8), which measures the amount of variability due to formation of new linkages or new chromosomal combinations within each cross, shows significant variation for all recombinant data, except when F values for double homozygotes are calculated. Chromosome III may be producing a complementary effect with II to account for this nonsignificant within variance. It is expected that within variance be significant for all recombinant chromosomes, because of the high number of new genotypes being formed by crossing over. As for non-recombinant chromosomes, chromosome III contrasts with II by showing a significant within crosses component. Chromosomal interaction for third chromosomes probably has a more drastic effect in lowering viability and producing more variation through interchromosomal action than chromosome II does.

*Lethals:* Lethals may arise in a population by mutation or recombination. In the process of testing viability values of 1,800 chromosomes in this experiment seven lethals were produced. Three lethals occurred on chromosome II (two occurred in the same cross following recombination, the other following nonrecombination). On the basis of known lethal mutation rates the production of lethals in this experiment could have arisen by mutation. Many investigators working on the origin of spontaneous lethal mutations on X chromosomes of *D. melanogaster* have agreed that between 0.1 and 0.4 percent of X chromosomes acquire a new lethal every generation. The longer chromosomes II and III have more loci than the X and it has been estimated that between 0.3 and 0.7 percent

of the second chromosomes acquire a new lethal by mutation every generation (see DOBZHANSKY 1951). Four lethals occurred on chromosome III (two each in crossover and noncrossover chromosomes). Figuring the average number of generations for the entire experiment (about 20) plus the observations on ten balanced strains over that time, the estimated approximate mutation rates for chromosomes II and III are 0.01 percent and 0.02 percent respectively per chromosome generation. This is a very much slower rate and is known to be characteristic of the Oregon-R strain.

Additional tests for three of the four lethals produced after recombination were made by SPIESS, CAPENOS and HELLING (1961). They were able to locate these lethals as point mutations on chromosomes II and III with the use of multiple recessive marker stocks.

A comparison of production of lethals in recombined chromosomes with those in nonrecombined chromosomes (4 to 3) shows no evidence for an excess production of lethals arising from recombination. This negative evidence applies for both second and third chromosomes for flies used in this experiment.

## DISCUSSION

If a sample of chromosomes is taken from a "well adapted" population and viability tests are run by making them homozygous, a nearly uniform result for good viability would be expected under the "classical" hypothesis, unless mutation is occurring at high rate. Repeatedly, investigations have shown this not to be the case. Indeed, chromosome samples taken from the laboratory population used in the above experiment exhibited a wide array of viability values for second and third chromosome when homozygous, including a high percentage of lethals (16 percent for chromosome II and 27 percent for III). During the 159 generations of its existence the population had acquired these lethals on second chromosomes, because the cage was started with lethal-free second chromosomes. Third chromosome lethals were almost twice as frequent. There is a good possibility that some lethals were present initially on the third chromosome since WALLACE had not eliminated these from that chromosome. After further tests by SPIESS, CAPENOS and HELLING (1961) it was discovered that several of the lethals on both chromosomes occurred in high frequency (4–8%). Therefore, this population is supporting lethals which would not be expected in a "classical" population unless mutation rates were exceedingly high or unless the breeding size of the population is small. High mutation rates can probably be ruled out since none of the lethals in the population were allelic with those produced by mutation in the present main experiment. While breeding size may account for occasional loci reaching frequencies as high as four percent, the non random clustered distribution of lethal loci found by SPIESS *et al.* (1961) would tend to indicate an active rather than passive function for such lethals, that is a selective advantage to lethals when heterozygous for certain loci.

WALLACE (1955) has shown that irradiated chromosomes, normally considered deleterious, have a slight heterotic effect on viability. On the other hand,

HIRAIZUMI and CROW (1960) demonstrated that heterozygotes for lethals and semilethals from a natural population have an appreciable deleterious effect on preadult viability (29 percent lethals or semilethals caused a reduction of 2.6 percent in preadult viability). This contradictory evidence seems to point out that there is no general rule for the effect of lethals in populations. Under one set of conditions lethals may show a heterotic effect, whereas under other conditions they may not, probably because heterosis may be produced for certain traits and not for others. The fact remains that lethals do exist at relatively high frequencies in the population from which material was drawn for this experiment. Then, if the population fits the "classical" scheme, it is probably very young in its evolution, and the rate of mutation for lethals exceeds their elimination or balances them or the population size is much smaller than estimated. Secondly, if the population fits the "balanced" scheme, it has incorporated lethals into its genetic structure and at least some of them have a heterotic effect. A third, and possibly the most realistic structure for the population, is a combination of "classical" and "balanced" schemes. Some loci are on their way to becoming fixed or eliminated in the homozygous condition while others have better fitness values in the heterozygous condition.

*Recombining "good viability" chromosomes*: To test the two schemes further, the effects of recombining "normal" chromosomes should indicate under which of the two schemes the population exists. As already stated "normal" chromosomes from a classic population would show little variation in viability after recombination. Ten "normal" second chromosomes, one subvital and nine "normal" third chromosomes from the population did show significant variation in viability values after recombination. However, the most extreme variation in viability, or the production of synthetic lethals, was not expressed.

The variation produced by recombination had the effect of increasing viability on chromosome II if non-recombinants are considered as controls. In contrast, SPASSKY *et al.* (1958), SPIESS (1959), and DOBZHANSKY *et al.* (1959) observed a constant lowering of viability after recombination in three wild species. It was proposed by these authors that the consistent change in viability is in the direction of average viability for homozygotes occurring in the original population. If that proposal is applicable to these results in *D. melanogaster* chromosome II, one could conclude that chromosomes were selected for the experiment at slightly lower viability than average second chromosomes in the population from which they were extracted. On the assumption that all lethals in the population were "point" rather than "synthetic" and therefore not relevant to the average recombinational effects of nonlethal chromosomes, the nonlethal population mean viability value ($32.8 \pm 0.84$) may be used as a reference for the recombined chromosome II value (33.2). Apparently the population mean is estimated slightly low. The increase of viability can be ascribed to epistatic interaction of recombined linked complexes since it did not occur with non-recombinant second chromosomes and since the net variance in viability within crosses was highly significant.

The experiments previously reported in this series did not include data from

non-recombinant chromosomes; assortment effects of whole chromosomes were not tested. When *D. melanogaster* chromosomes III and II together with III were tested, viability was reduced equally by whole chromosome reassortment and by intrachromosomal recombination. This lowering effect consequently must arise principally from interchromosomal activity. This fact raises the question as to how much of the viability lowering in the previous experiments on the wild species could be accounted for by genetic background interactions and how much by intrachromosomal linkage exchanges. The large autosomes should assort independently; it would hardly be expected that mechanisms to prevent random assortment would exist to keep "good" interactions together. Consequently it must be re-emphasized that these viability measurements have been made on homozygotes which probably never exist in nature or in the laboratory WALLACE population; selection must favor high fitness among the interactions between non-homologous chromosomal heterozygotes. Deleterious viability of homozygotes then can result from either "poor" linkage combinations or "poor" interchromosomal combinations or both.

The greater average lowering of viability is relatively apparent in the non-recombinant double homozygotes for chromosome II and III. When considered separately both non-recombinant II and III had shown the effect, though II was not quite significant. The two together not only reflect reinforcement of the data for each chromosome by their parallel tendencies, but very well indicate that new chromosomal interactions not selected for in the original strains may have a substantial deleterious effect. If the chromosomes are segregating and assorting at random, isoalleles from non-recombinants will segregate one half of the time into combinations identical with those of each strain and the other half of the time into new combinations. Presumably the lowering effect would be still greater if the new combinations alone could be isolated and measured.

The intermediate viability values for recombinant double homozygotes of course reflects the opposite tendencies of chromosomes II and III when taken separately. It also may reflect the fact that with crossing over on both chromosomes most combinations of new linkages would be present in some portion of the genetic background in which they were originally selected.

The analyses of variance (Tables 7 and 8) support the conclusion that both polygenic and chromosomal interaction accounts for production of variation, which vary in amount and type of interaction depending on the chromosome in question. Chromosome III is apparently sensitive to its background and shows adaptive properties with certain background genotypes, e.g., the background of such strains used in this experiment were suitable for "good" viability, but once a new background was introduced by crossing strains the result was a general lowering of viability. This is supported by the significant F value for segregating unrecombined third chromosomes. Chromosome III also shows a significant F value for interaction of polygenes to produce variation and lower viability by crossing over but to a lesser extent than chromosome II. On the other hand chromosome II contrasts with III in producing less variation through interchromosomal interaction as seen by a comparison of F values for segregating unrecom-

bined and recombined second and third chromosomes.

If segregating whole chromosomes are producing more variation than recombined linked complexes there should be significant within variance in unrecombined chromosomes. This was true only for chromosome III ($P = 1\%$). Linked complex recombination was sufficient in chromosome II to produce a within significant variance (2.5–1.0%), but not for whole chromosomal interaction. These findings show a clear difference between chromosomes II and III.

*Chromosomal and polygenic interaction from "normal" chromosomes in relation to the original population:* The ten strains chosen for recombinational studies with pertinent data for comparison with the original population are summarized in Table 9. Standard errors (between means of strains, crosses, or original chromosome viabilities) are given for convenience. Real variances ($V$) for controls were negative, that is effectively zero. The variances for populational chromosomes can be compared with variances engendered by recombination as follows: Recombination of second chromosomes increases variability up to 42 percent (23.68/56.25) of that observed for nonlethal chromosomes in the original population. The variance values for whole second chromosome assortment are not raised significantly (see Table 8) above residual variance, in contrast. Therefore segregation of second chromosomes into different genetic backgrounds may have relatively little effect in producing variability in the original population.

Recombination of third chromosomes increases variance up to 96 percent (27.71/28.94) of that observed for nonlethal chromosomes in the original population. In addition the assortment of whole third chromosomes produces variance significantly greater than residual variance (Table 8), and the amount increased comes to 51 percent (14.77/28.94) of that observed in the original population.

Mutations could be producing some of the variation in this population. It is expected that the rate would be the same in all chromosomes tested; control and experimental chromosomes should have equal mutation rates. There is little evidence for a high rate of mutation (especially for lethals) on either second or third chromosomes in this population, judging by the fact that only one lethal mutation (4) was detected in strain cultures during the entire experiment. No evidence of polygenic mutations can be drawn from control counts made at the

TABLE 9

*Comparison of the original population with experimental after crossing. Lethals were excluded. S.E. = standard deviation of cross or strain means;* V *= real variances within crosses;* $\sigma^2$ *= variance of nonlethal original chromosomes*

| | Original population | | | | Experimental chromosomes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Before crossing | | After crossing | | | |
| | Mean viability | N | S.E. | $\sigma^2$ | Mean viability | S.E. | Mean viability | | S.E. | $V$ |
| II | 32.3 | 80 | ±0.84 | 56.25 | 32.3 | ±0.77 | Rec. | 33.2 | ±0.71 | 23.68 |
| | | | | | | | Non-rec. | 30.5 | ±0.37 | 6.94 |
| III | 28.2 | 63 | ±0.68 | 28.94 | 30.6 | ±0.94 | Rec. | 28.3 | ±0.62 | 27.71 |
| | | | | | | | Non-rec. | 27.9 | ±0.48 | 14.77 |

end of the experiment. There is, therefore, no reason to assume that experimental chromosomes mutated at higher rates than the controls or that mutation was greater on either chromosome.

From these comparisons the conclusion can be drawn that carefully chosen normal viability second and third chromosomes may release vast amounts of variation by crossing over up to almost all that observed in the case of chromosome III. This released variation is relatively more available after crossing over in second chromosomes than after reassortment, while in third chromosomes nearly equal amounts of variation are released by reassortment of whole chromosomes as by crossing over.

Average viability is increased slightly by recombination of chromosome II. Both recombination and whole chromosome assortment lower viability on chromosome III in contrast with the second chromosome but in each case the population average is probably approached.

It is important to note that this difference between chromosomes may reflect the manner in which the original population was constructed and the constant laboratory environment in which the population was maintained; deleterious second chromosomes were eliminated while third chromosomes were unselected by WALLACE, and the population experienced constant temperature and food conditions. In spite of numerous deleterious mutations which have evidently been incorporated into the total gene pool for the second chromosome, its nonlethal average viability remains high after recombination. Certainly heterozygosity must be in high frequency; additive effects after recombination are not significant; so that the conclusion must be that a basic assumption of WALLACE *et al.* (1953) that "unselected combinations of alleles would often produce ill-adapted recombination products" is not the inevitable result of balanced heterozygosity. Apparently ill-adapted recombination products are not the necessary consequences of high heterozygosity. Another possibility is that the heterozygosity is not balanced in this case, however that possibility is not likely as can be inferred from the high viability of strain cross $F_1$ viabilities (34.8 for chromosome II and 32.4 for chromosome III), which are higher than all homozygous averages measured in the experiments reported here.

It is likely therefore that the original population second chromosomes have relatively few genic complexes which are markedly deleterious in homozygous condition. Such deleterious genotypes that do exist on that chromosome are more likely to be single recessive loci but probably represented by many isoalleles arisen by mutation since the initiation of the population. Conversely, WALLACE in selecting highly fit second chromosomes was with respect to third chromosome selecting good interchromosomal effects. These effects should be stabilized by selection in the population, under conditions of heterozygosity. For this chromosome, homozygosity coupled with reassortment into new genetic backgrounds is deleterious. In other words it is far more likely that these chromosomal characteristics are a product of the population's genetic architecture and selection scheme than they are intrinsic properties of the species' two large autosomes. One might hazard a prediction that if the original population had been estab-

lished by clearing deleterious genotypes from chromosome III initially instead of from chromosome II, the recombinational effects of the two chromosomes might have been reversed. Possibly the constancy of environment has not created demands on chromosome II to bring about a response for high plasticity as it might in a fluctuating wild environment. Recombinational studies on wild populations of *D. melanogaster* would be needed for a comparison.

It cannot be concluded then that chromosomes II and III differ beyond the confines of this experiment. Comparisons between several populations would have to be made to establish such a fact. The negative results of HILDRETH in not being able to find synthetic lethals on chromosome III seem reinforced by this study, but it should be kept in mind that only ten chromosomes of good viability were sampled. A larger sample or one with subvitals or chromosomes which can engender widely divergent viabilities might produce lethals by crossing over or by interchromosomal effects. WALLACE (1953) curiously enough got the preponderant portion of his lethals from crossing over between subvitals (his strains 4, 5, d, and e). It is probably true therefore that "good" viability chromosomes are less likely to produce extremely deleterious viabilities by crossing over than chromosomes of subvital viability. If that subvitality depends on an interaction between many loci, other linkages of those loci might conceivably be even worse, namely lethal. The matter is still far from solved but the direction of the problem is becoming well-defined.

## SUMMARY

Viability tests on second and third chromosomes of 100 males from a laboratory population of *D. melanogaster* indicated values ranging from lethal to supervital, with a higher frequency of lethals and lower average viability on chromosome III. The population had been initiated with lethal free second chromosomes by WALLACE and had been maintained for more than 159 generations.

Ten strains with "good viability" homozygous second and third chromosomes to be used for recombinational studies were chosen from the 100 males tested. Each strain was crossed with all other strains and the effects of crossing over in $F_1$ females, as well as the effects of whole chromosome assortment by the use of $F_1$ males, were observed (by testing 450 recombinant and non-recombinant second and third chromosomes for a total of 1,800 chromosomes) by producing homozygosity with special marker techniques.

The average viability was increased by recombination in homozygous second chromosomes but decreased in homozygous third chromosomes and double homozygotes. Reassortment of whole chromosomes acted to decrease viability in third chromosomes and double homozygotes presumably by producing newly assorted nonselected combinations of chromosomes.

Intrachromosomal recombination produced more variation in viability values than did interchromosomal recombination. However, reassortment produced more variation than the "normal viability" original strains. Comparisons with the t test and analyses of variance show that variation produced by crossing over

(variance within crosses) is always significantly greater than residual variation, but that produced by chromosomal assortment is significant only for chromosome III. No additive effects were significant for either chromosome or double homozygotes. Nonadditive (interaction) variances are significant for both chromosomes and double homozygotes.

There seems to be a real genetic difference between second and third chromosomes in their response to recombination and segregation of unrecombined chromosomes. Recombination engenders significant variation for both, but increases viability in II and lowers it in III. Chromosome III is apparently more sensitive to its genetic background than II as deduced by the increased chromosomal interaction of the former. This difference may, however, reflect the initial composition of the population.

A very low rate of lethal mutation was observed in experimental strains and no more lethals were produced in crosses with recombination than without recombination. Therefore, there is no evidence for the production of synthetic lethals from "normal viability" second and third chromosomes in this experiment. Possibly synthetic lethals are more likely to be produced from recombination of subvitals than from good viability chromosomes.

It was concluded that genetic recombination and segregation of whole chromosomes produce variation in viability that reverts selected homozygous chromosomes toward the nonlethal average viability observed in the original highly variable population. Intrachromosomal recombination restores 40 percent or 95 percent of the variation observed for chromosomes II or III respectively while interchromosomal recombination of chromosome III accounts for 50 percent of the original variation of that chromosome.

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# FURTHER DATA ON NON-HOMOLOGOUS PAIRING AND CROSSING OVER IN DROSOPHILA MELANOGASTER

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IT was previously reported that *Drosophila melanogaster* females heterozygous for the sex-linked, recessive white mutants $w^a$ (white-apricot) and $w^{a2}$ (white-apricot-2) infrequently but regularly produce in association with crossing over an X chromosome deficient for the salivary gland chromosome bands 3A4 to 3C1 inclusive (GREEN 1959a). To explain the origin of the deficient X chromosome, it was suggested that a comparatively high degree of synaptic affinity exists between band 3C1, part of the white pseudoallelic array, and band 3A3, the zeste ($z$) locus (GANS 1953). Pairing at meiosis between 3A3 and 3C1 accompanied by a crossover to the immediate right should produce both a deficiency of the limits noted above and a recriprocal duplication in which the segment equivalent to the deficiency is duplicated in tandem. A convincing demonstration of the plausibility of this interpretation requires the recovery of both the deficiency and duplication. While the deficiency was readily recovered, the duplication was not. Experiments which led to the recovery of the duplication together with some of its phenotypic properties will form the substance of this report.

## EXPERIMENTAL

The experiment which successfully led to the recovery of the tandem duplication was predicated on a number of observations. GANS (1953) clearly established the dependence of $z$ upon the white region for its manifestation. Subsequent information (GREEN 1959a) suggested that of the salivary gland chromosome bands 3C1, 2 and 3, which collectively constitute the white region, in all probability only 3C1 is necessary for the phenotypic expression of zeste. This was supported by the recovery of an X chromosome carrying zeste but deficient for the intervals 3A4–3C1 in the following experiment.

Among the progeny of females *sc z* $w^a$ *spl*/$w^{a2}$; *Cy*/+; $Ubx^{130}$/+ crossed to *y w spl* $sn^3$ males (*sc* = scute bristles, *spl* = split eye and bristles, *y* = yellow body, $sn^3$ = singed-3 bristles, *Cy* = Curly wings, $Ubx^{130}$ = Ultrabithorax halteres) five exceptional white-eyed females were found. Four were *spl* and subsequently shown not to carry *sc* while the fifth was not *spl* and proved to carry *sc*. All females produced only *y w spl* $sn^3$ males establishing the male lethality of the homologous X chromosome. The deficiencies associated with both the *sc* and *spl* marked X chromosomes were shown by genetic tests described in detail elsewhere (GREEN 1959a) to lack band 3C1 but not bands 3C2,3 and to extend as

far to the left of 3C1 as band 3A4. For simplicity the two deficient chromosomes will be designated $w^-$ *spl* and *sc* $w^-$. Their origin may be explained by crossing over subsequent to pairing between the zeste locus and 3C1 as described above. In the case of the $w^-$ *spl* chromosome pairing most likely occurred between $z^+$ of the $w^{a2}$ X chromosome and 3C1 of the $w^a$ chromosome. On the other hand for the *sc* $w^-$ deficiency pairing occurred between *z* and $w^a$ chromosome and 3C1 of $w^{a2}$ chromosome. That the *sc* $w^-$ chromosome also carries *z* was established as follows. By crossing over, *z* was introduced into an X chromosome carrying a tandem duplication of the entire white region (GREEN 1959a) and designated *Dp(1)*$w^a$. From the appropriate crosses, females of the genotype *sc* $w^-/z$ *Dp(1)*$w^a$ and $w^-$ *spl*/*z Dp(1)*$w^a$ were obtained. The eye phenotype of the former was zeste and of the latter non-zeste demonstrating that the *sc* $w^-$ chromosome carried the *z* mutant and the $w^-$ *spl* chromosome the $z^+$ allele. These findings are consistent with the pairing and crossing over scheme postulated as the source of the $w^-$ chromosomes.

By appropriate crosses, females of the genotype *sc z* $w^-/z$ $w^+$ were obtained. Their $z^+$ eye color demonstrated the necessity of a double dose of band 3C1 for the phenotypic manifestation of *z*.

Two observations led to the experiment designed to recover the tandem duplication reciprocal to the $w^-$ chromosomes. First, as a by-product of X-raying males of the genotype $y^2$*su–*$w^a z$ for a different purpose, a duplication of *z* was recovered not unlike those described by GANS (1953). In this duplication, herein designated *Dp(1)z*, the zeste mutant is apparently present twice but the white region is unaltered. A salivary gland chromosome analysis kindly made by DR. EILEEN S. GERSH confirmed the duplication nature of *Dp(1)z* and suggests that bands 3A1-4 are duplicated. Females *Dp(1)z/z* or *Dp(1)z* homozygous are zeste in phenotype while males *Dp(1)z* are wild type just as are *z* males, and this is expected since the white locus is unduplicated. Second, females of the genotype *Dp(1)z/z*$^+$*Dp(1)*$w^a$ are zeste in eye color. From this fact it was reasoned that if band 3C1 is needed to evince the zeste phenotype, the duplication complementary to the $w^-$ chromosome should be a duplication of 3C1, and should when compounded to *Dp(1)z* yield zeste colored females. Hence the duplication of 3C1 should be recovered among the female progeny of the cross $w^a/w^{a2}$ females by *Dp(1)z* males.

Accordingly females identical in genotype to those among whose progeny the $w^-$ chromosomes were first found, i.e. $y^2$ *su–*$w^a$ $w^a$ *spl*/$w^{a2}$; *Cy*/+; $Ubx^{130}$/+, were obtained, crossed to *sc Dp(1)z* males, and zeste females were sought. Among approximately 30,000 female progeny two manifesting a zeste eye color were found. Since in earlier experiments with an identical marker arrangement, the great majority of the $w^-$ chromosomes recovered also carried the *spl* marker, the duplication should be marked $y^2$ *su–*$w^a$ $w^{a2}$. The following pairing scheme, with the vertical arrows indicating the point of crossing over, describes the origin of both recombinants.

$$y^2\ su\text{–}w^a \ldots\ldots z^+ \ldots\ldots 3C1 \downarrow w^a \ldots\ldots\ldots\ldots\ldots spl$$

$$z^+ \uparrow \ldots\ldots\ldots 3C1\ w^{a2} \ldots\ldots\ldots$$

Therefore on progeny testing the two zeste females should produce two types of males: those *sc Dp(1)z* and those *y² su–w^a w^a²*. These predictions were borne out, and it was concluded that the *y² su–w^a w^a²* males possess a tandem duplication for the interval 3A4-3C1, a duplication which for simplicity will be designated *Dp(1)3C1*. A cytological examination kindly made by DR. GERSH shows a tandem duplication present, but its precise limits are not clear except that 3C1 is duplicated.

If the genetic constitution of *Dp(1)3C1* is as indicated and if the zeste phenotype depends primarily upon a double dose of 3C1, males of the genotype *z Dp(1)3C1* should have a zeste eye color. Such males, zeste in eye color, have been readily obtained following crossing over in females of the genotype *z/y²su–w^a Dp(1)3C1*. Furthermore, females of the genotype *sc z w⁻/z Dp(1)3C1* are zeste in eye color, an effect consistent with the idea that a double dose of band 3C1 is necessary for the expression of the zeste phenotype.

Two types of white mutants have been described (GANS 1953; GREEN 1959b). One type, including such mutants as *w^e*, *w^ch*, *sp–w*, act as dominant suppressors of zeste. Mutants of the second type, of which *w^a*, *w^bf*, *w^co* are representative, do not alter the zeste phenotype. Females of the genotype *z w^e/z Dp(1)3C1* are zeste in eye color showing that the extra 3C1 band counteracts the suppressing effect of *w^e*. This action of *Dp(1)3C1* apparently does not directly involve the role of *w^e* in eye pigment synthesis since males *Dp(1)3C1 w^e* appear to be phenotypically identical to regular *w^e* males. A comparable effect of *Dp(1)3C1* was found when tested with the mutant *w^11G3*. This X-ray-induced mutant, which lacks a characteristic phenotype of its own, was found to be localized to the white region and to act as a dominant suppressor of zeste (GANS 1953). Females of the genotype *z w^11G3/z Dp(1)3C1* are typically zeste in eye color.

The wild-type phenotype of females compounded of zeste and any of a wide variety of white mutants indicates nonallelism and accordingly "functional" dissimilarity. That this conclusion is an oversimplification is suggested by the eye phenotypes manifest under specified genetic conditions where either *Dp(1)z* or *Dp(1)3C1* are involved. While a wild-type eye color is characteristic of females heterozygous for *Dp(1)z* and any one of five separate zeste suppressing white mutants, equivalent compounds in which any one of eight nonsuppressing white mutants was used are clearly not wild type, but manifest a light to dark maroon eye color, more or less variegated, depending upon the *w* mutant. Comparable findings are noted when *Dp(1)3C1* is employed and differential phenotypes in compounds with *w^a* and *w^e* are representative. Females of the genotype *z Dp(1)3C1/w^e* are wild type, but females *z Dp(1)3C1/w^a* manifest a maroon eye color with noticeable variegation.

Of some interest are observations made on the effect of *Dp(1)3C1* on the specificity of *su–w^a*. Earlier studies (SCHULTZ, personal communication; GREEN 1959b) showed that among a wide range of white mutants studied, this suppressor affected only *w^a* and some partial back mutations of *w^a*. The first suggestion of nonspecificity of *su–w^a* came with the recovery of males *y² su–w^a Dp(1)3C1 w^a²*. These have a distinctly darkened eye color not unlike that of *su–w^a w^a* indi-

viduals. By crossing over it was readily shown that the altered $w^{a2}$ eye color was not due solely to the duplication since *Dp(1)3C1* $w^{a2}$ males were phenotypically inseparable from regular $w^{a2}$ males. When *su–w*$^a$ was restored to the *Dp(1)3C1* $w^{a2}$ chromosome by crossing over, the altered $w^{a2}$ phenotype recurred. Subsequent crossing over experiments were carried out to determine the combined effectiveness of *Dp(1)3C1* and *su–w*$^a$ on mutants pseudoallelic and allelic to $w^a$ and $w^{a2}$ which are allelic. The mutants selected were $w^e$ localized to the right of $w^a$ and $w^{a2}$, $w^{col}$ localized to their left and $w^{a4}$ allelic to $w^a$. Individuals in which $y^2$ *su–w*$^a$ *Dp(1)3C1* were recombined with each of the white mutants were obtained and their eye phenotypes in all instances were suppressed. These data demonstrate the relative nonspecificity of *su–w*$^a$.

## DISCUSSION

Implicit in any evaluation of the combined data presented earlier (GREEN 1959a) and here is the question of the utility of the several operational criteria employed in assigning the genetical relationships among independent gene mutations, and specifically the genetical relationships between the zeste and white loci. Functional properties of genes, whatever function connotes, are especially difficult to assign in the absence of detailed descriptions of specific biochemical (enzymatic) alterations associated with particular mutants. Where by necessity phenotypic criteria form the sole basis for discerning functional relationships, ambiguities inevitably arise. This is strikingly illustrated in the case of the zeste and white mutants. The dependence of the zeste phenotype upon a double dose of band 3C1 has been demonstrated, thereby establishing a "functional" relationship between *z* and band 3C1. That 3C1 forms an integral part of the white region follows from the fact that any white mutant manifests pseudodominance when compounded with deficiencies for this band just as with deficiencies for either the 3C2,3 doublet or for all three bands. While this information implies functional relationship, other data point to functional divergence. Thus individuals homozygous for 3C1 deficiencies or for 3C2,3 deficiencies are inviable, yet the causes of lethality must be different for the two since females compounded of the two deficiency types, i.e. 3C1 deficiency/3C2,3 deficiency are viable (GREEN 1959a). In addition there is evidence that functional divergence exists between bands 3C2 and 3C3. It is well known that two types of white mutants occur: those suppressing zeste; those that do not (GANS 1953). A recombination analysis showed that the zeste suppressors lie to the right of the nonsuppressors (GREEN 1959b) with the latter localized in all probability to band 3C2, the former to 3C3. It is obvious from these facts that no clear-cut, simple picture of the functional attributes of the zeste and white regions is possible from these data. That functional interdependence occurs is obvious; the precise underlying mechanisms are not.

Any attempt to assign genetical relationships between zeste and the components of the white region must take into consideration pairing or synaptic behavior. If, as generally believed, pairing is either a prerequisite to or concomitant

with crossing over, the recovery of the duplication and deficiency of region 3A4–3C1 is *prima facie* evidence for some measure of synaptic affinity between 3C1 and the zeste locus. If the frequency of recovery of recombinants is, in part, a measure of the degree of the effective pairing (PRITCHARD 1960) which occurs between loci, it follows that under the genetical conditions described, pairing between the zeste locus and 3C1, as measured by the frequency of the duplication and deficiency recombinants, occurs as frequently as pairing between allelic doublets 3C2,3 as measured by the frequency of crossing over between the pseudoalleles $w^a$ and $w^{ch}$. Insofar as it is currently possible to judge, 3C1 exhibits a higher degree of synaptic affinity for the zeste locus than for either half of the 3C2,3 doublet.

How can the concept of locus be best assigned to white? It has been argued primarily from studies of pseudoallelism that the locus defined in terms of crossing over is outmoded. In its place the locus (gene) can be defined as a unit of varying genetical length whose component parts, separable by crossing over, manifest a coordinated function directed to the synthesis of a single protein or polypeptide. While it is not possible to describe the function of zeste and white in terms of a specific biochemical synthesis, their phenotypic interactions as described are not incompatible with the idea that they are uniquely associated in a common biosynthesis. This means that zeste and white are components of a single functional locus made up of approximately 15 salivary chromosome bands, extending from 3A3 to 3C3 inclusive, and whose genetical length is equal to about 0.6 percent crossing over. Within this region at least one other gene has been described, an enhancer of bithorax with an eye color effect (LEWIS 1959) whose relationship to zeste and white is not clear.

Finally since zeste and 3C1 exhibit a high degree of homology, however defined, what has heretofore been designated non-homologous requires correction.

## SUMMARY

1. The recovery is described of a tandem duplication extending from salivary gland chromosomes bands 3A4–3C1 and reciprocal to a previously reported deficiency.

2. Evidence is presented showing that the zeste mutant depends upon band 3C1 for its manifestation.

3. The concept of the gene locus is discussed, and it is concluded that upon applying available criteria, zeste and white should be considered as part of the same locus.

## ACKNOWLEDGMENT

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